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Characterization of phytochemicals stimulatory to sexual reproduction in *Phytophthora cactorum* and *P. parasitica*

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Abstract. *Phytophthora cactorum* and *P. parasitica* formed oospores on basal medium supplemented with corn oil or crude soybean lecithin. The non-saponifiable fraction from these two supplements were stimulatory to oospore formation of both fungi, but the saponifiables were stimulatory to only *P. cactorum*. When the non-saponifiables were subjected to Florisil column chromatography, the fraction eluted with 25% diethyl ether in hexane was the most stimulatory. Thin layer chromatography (TLC) separated this fraction into three active bands. Among them, the two non-digitonide TLC bands were further separated by high performance liquid chromatography (HPLC), and the active fractions were isolated for gas chromatography-mass spectrometry (GC-MS) analysis. Compounds identified were 3-eicosyne, β-farnesene, tetradecane (2, 6, 10-trimethyl), docosane, hexadecanol (2-methyl), phytol, and pentatriacontane. Commercially available phytol tested was found to be highly stimulatory to oospore formation of both fungi. It was active even at the concentration of 1 ng/basal medium disc.

Keywords: Corn oil; *Phytophthora cactorum*; *Phytophthora parasitica*; Phytol; Sexual reproduction; Soybean lecithin.

Introduction

Sterols were considered to be required for sexual reproduction in pythiaceous fungi with the homothallic *Phytophthora cactorum* (Lebert and Cohn) Schroeter as the most extensively studied organism. This requirement was believed to be caused by the inability of these fungi to perform sterol synthesis (Hendrix, 1970; Elliott, 1983). However, it was subsequently found that sterols were stimulatory but not essential for sexual reproduction in *Phytophthora cactorum* because the process could also be induced by phospholipids (Ko, 1985; Ko and Ho, 1983). Furthermore, lecithins but not sterols were also stimulatory to the sexual reproduction of heterothallic *Phytophthora parasitica* Daster and *Phytophthora capsici* Leonian (Ko, 1985; Ko and Ho, 1983). Some glycerides such as dipalmitin were also stimulatory to sexual reproduction in *Pythium aphanidermatum* (Edson) Fitzpathrick (Ko, 1986). Recently a number of highly purified commercial fatty acids and related compounds were found to contain substances inhibitory to oospore formation of *P. cactorum* (Jee et al., 1997; Jee and Ko, 1997). After removal of the inhibitory substances, most of these compounds became stimulatory to the formation of oospores in *P. cactorum*. Palmitoleic acid, phytol, retinol, and vitamin A esters were also stimulatory to the sexual reproduction of *P. parasitica* (Jee and Ko, 1997).

Although *P. cactorum* is incapable of synthesizing sterols, the extract from its mycelia grown in liquid basal medium was stimulatory to oospore formation of both *P. cactorum* and *P. parasitica* (Jee and Ko, 1998). The stimulatory substances synthesized by pythiaceous fungi included various types of tepenoids, fatty acids, phospholipids, and other lipids (Losel, 1988; Jee et al., 1997; Jee and Ko, 1997). Growing in basal medium, this group of fungi requires a stress factor such as nutrient deprivation or limitation of growth to trigger the process of sexual reproduction (Jee and Ko, 1998). The stress factor is not needed when certain commercial organic compounds such as phospholipids, fatty acids, and sterols are added (Elliott, 1983; Jee et al., 1997; Jee and Ko, 1997).

Since oospores are commonly produced in plant tissues by homothallic pythiaceous pathogens (Agrios, 1988), host plants must contain substances stimulatory to sexual reproduction in these organisms. A project was, therefore, initiated to characterize these substances. Corn oil was used in this study because vegetable oils are known to be stimulatory to sexual reproduction of pythiaceous fungi (Haskin et al., 1964; Klemmer and Lenney, 1965). Crude

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soybean lecithin was much more effective than highly purified lecithin in inducing oospore formation of *P. cactorum* and *P. parasitica* (Ko and Ho, 1983). It was, therefore, also included in this study to determine if substances other than lecithin are stimulatory to the oospore formation of these two fungi.

Materials and Methods

Organisms

Isolate 121F of *P. cactorum* was supplied by Dr. D. L. McIntosh (Research Station, Agriculture Canada, Summerland, BC, Canada). The A1 mating type (isolate P991) of *P. parasitica* was supplied by Dr. G. A. Zentmyer (Department of Plant Pathology, University of California, Riverside, CA) while the A2 mating type (isolate 6134) of the same species was isolated from eggplant fruit (Ann and Ko, 1988). All cultures were derived from single zoospores.

Basal Medium

Modified basal medium of Ko and Ho (1983) was used for maintenance of test organisms and for bioassays as previously described (Jee et al., 1997). The basal medium consisted of 0.1 g KNO_3, 0.2 g K_2HPO_4, 0.1 g $MgSO_4$, 0.1 g $CaCl_2$, 1 ml trace elements, 0.1 g L-asparagine, 0.05 g L-serine, and 4 g glucose in 1 l of distilled water. The trace element solution contained 200 mg of FeEDTA, 10 mg $CuSO_4$, 10 mg $MnCl_2$, 10 mg Na_2MoO_4, 10 mg $Na_2B_4O_7$, 2 mg $ZnSO_4$, and 100 mg thiamine hydrochloride in 100 ml of distilled water. Highly purified SeaKem agarose (HGT-P Agarose, FMC, BioProducts, Rockland, ME, USA), which did not contain detectable nutrient contaminants (Ho and Ko, 1980; Ko, 1985), was used to solidify the basal medium at a concentration of 0.8% (w/v). The medium was adjusted to pH 6.2 with 0.5 N KOH. The modified basal medium supported better mycelial growth of the test organisms than the original basal medium. For each test organism, a strip (ca 3 × 3 × 40 mm) of culture grown on 10% V-8 agar (10% V-8 juice, 0.02% $CaCO_3$, and 2% agar) was placed in the center of a culture plate. After incubation at 24°C for 14 d, the same sized culture strip was cut 20 mm away from the inoculum and placed in the center of a plate containing fresh basal medium. The procedure was repeated a minimum of ten times before use, to avoid possible contamination of stimulatory substances from the original inoculum. Under such conditions, single isolates of *P. cactorum*, or paired A1 and A2 isolates of *P. parasitica*, did not form oospores on the basal medium unless a stimulatory substance such as lecithin was added.

Bioassay

Six basal medium discs (12 mm diam, 5 mm thick) were evenly distributed in a Petri plate and 10 µl of ethyl acetate solution containing 10 or 100 µg of a substance to be assayed was spread over the surface of a basal medium disc. After evaporation of ethyl acetate in a fume hood for 10 min, the basal medium disc was inoculated by placing on its top a small cube (ca 2 × 2 × 2 mm) of *P. cactorum* culture, or cubes of A1 and A2 strains of *P. parasitica* separated from one another by 2-3 mm. Inoculated discs of basal medium in Petri plates were incubated for 7 d at 24°C in darkness in a moist chamber for oospore production. To determine the number of oospores produced, three discs were triturated with 15 ml of distilled water in an Omni mixer at 4,000 rpm for 1 min. Oospore concentration in the suspension was determined by counting the number of oospores in a 10 µl or 1 ml sample (Ko et al., 1973). Immature or aborted oospores were excluded from the calculation of spore concentration. Three replicates were used for each treatment, and all experiments were repeated at least once.

Saponification of Corn Oil or Crude Soybean Lecithin

Commercial corn oil (Hunt-Wesson, Inc., Fullerton, CA 92834) and crude lecithin (soybean, refined, oil removed, 95% acetone insolubles, 1% free fatty acid, 9% ash, 1% moisture, ICN Biochemicals, Irvine, CA 92713) were saponified following the Official Method of the American Oil Chemists' Society (1971). Fifty g of corn oil or crude lecithin was added to 50 ml of 50% KOH and 300 ml of 95% ethanol in a 1000 ml boiling flask. The mixture was gently boiled under reflux condenser for 1 h, and adjusted to 800 ml with deionized water. Non-saponifiable compounds were extracted by 3 aliquots of 500 ml of petroleum ether. The petroleum ether portion was evaporated in a rotary evaporator. The residue was dissolved in 40 ml of acetone and stored overnight at -20°C to precipitate and remove some sterols and residual phospholipids (Kates, 1986). The clear solution was referred to as the non-saponifiable fraction. The saponifiable fraction was obtained by adjusting the aqueous portion to pH 2 with 6N HCl and extracting the acidified solution with petroleum ether. Both non-saponifiable and saponifiable fractions were evaporated, and the residues were redissolved in ethyl acetate before bioassay

Florisil Column Chromatography of Non-Saponifiable Compounds

Chromatography of non-saponifiable compounds on Florisil was performed following the method of Carroll (1961). Thirty g of Florisil (60-100 mesh, Fisher Scientific, Pittsburgh, PA 15219) was packed in a column (2 × 30 cm) with hexane, and 300 mg of the non-saponifiables in 3 ml of hexane was loaded. The column was eluted with solvents in the following order: 60 ml of hexane, 200 ml of 5% diethyl ether in hexane, 200 ml of 25% ether in hexane, and finally 200 ml of 2% methanol in ether. Each fraction was evaporated, and the residue was dissolved in ethyl acetate before bioassay. For further treatment, the fraction eluted with 25% ether in hexane was evaporated in a rotary evaporator. The residue was dissolved in 5 ml of acetone for crystallization at -20°C to remove sterols as described above. The clear solution was evaporated, and the residue was dissolved in ethyl acetate.

Thin Layer Chromatography (TLC)

The 25% ether-hexane fraction dissolved in ethyl acetate (20 mg/ml) was spotted on a TLC plate (10 × 20 cm, 60 A silica gel, 1000 µm layer thickness, Whatman adsorption plate, Fisher Scientific, Pittsburgh, PA 15219) using 5 µl per spot. The loaded plate was developed with hexane-ether-acetic acid (70:30:1) and visualized with iodine vapor stain (Kates, 1986). After evaporation of iodine, each band was scraped off from the plate and extracted with two aliquots of 20 ml ether.

Digitonin Treatment of the Compounds Recovered from TLC

The procedures used by Hendrix (1966) and Kates (1986) were followed with some modifications. Each fraction (ca. 5 mg) was dissolved in 2 ml of acetone-95% ethanol (1:1) and mixed with 2 ml of 1% digitonin. The digitonin solution was prepared by dissolving 1 g of digitonin in 50 ml of 95% ethanol and adjusting the solution to 100 ml with deionized water. After incubation at 24°C for 2 h, the mixture was centrifuged at 5,000 rpm for 10 min. Supernatant and precipitate were collected and referred to as the non-digitonide fraction and the digitonide fraction, respectively. The non-digitonide fraction was extracted with 20 ml of ether, and the extract was washed with 40 ml of 1% NaCl solution, recovered, and evaporated to dryness. The residue was dissolved in ethyl acetate for bioassay. The digitonide fraction was mixed with 5 ml of acetone-ether (1:1) and centrifuged to remove non-digitonide contaminants. The washing process was repeated, and the washed digitonide was dissolved in 2 ml of pyridine to free the putative sterols from the digitonin complex and washed with 20 ml of 1% NaCl. The putative sterols were extracted from the mixture with two aliquots of 10 ml of ether.

High Performance Liquid Chromatography (HPLC)

The most active non-digitonide fractions of TLC bands 1 and 2 from corn oil were further fractionated on HPLC (Beckman, Model 110) using a C18 column (Radial-Pak C18, Waters RCM, 8 × 10, Millipore). Compounds were detected at 210 nm. Twenty µl of sample (20 µg) in ethyl acetate was injected into the column and eluted isocritically with methanol-acetonitrile-water (46: 46: 8) at l ml/min for 40 min. All significant peaks were collected separately. Each fraction obtained from five injections was evaporated, and the residue was dissolved in 200 µl of ethyl acetate for bioassay or further analysis.

Gas Chromatography (GC) and Gas Chromatograph-Mass Spectrometry (GC-MS)

Fractions obtained from HPLC in ethyl acetate were separately injected into an HP 5890 gas chromatograph equipped with a flame ionization detector and a DB-5 capillary column (30 m × 0.25 mm, J & W Scientific, Folsom, CA 95630). The flow rate of helium carrier gas was 1 ml/min. The column temperatures were 60°C for 2 min, increased at 15°C/ min for 10 min, and maintained at 210°C for 5 min. The temperature was increased again at 10°C/ min for 8 min and maintained at the final temperature of 290°C for 25 min. For GC-MS an HP 5790 A instrument interfaced with HP 5890 GC fitted with a DB-5 column was used.

Results

Fractionation of Corn Oil and Soybean Lecithin by Saponification

Phytophthora cactorum and *P. parasitica* did not form oospores on basal medium unless corn oil or crude soybean lecithin was added (Table 1). Soybean lecithin was more effective than corn oil in inducing oospore formation of both fungi. Saponification of 50 g of corn oil generated 45.7 g of saponifiables but only 0.44 g of non-saponifiables. However, saponifiables were less active than non-saponifiables. Moreover, the latter were stimulatory to oospore formation by both *P. cactorum* and *P. parasitica* while the former were stimulatory to oospore formation by *P. cactorum* but not *P. parasitica*. After saponification, 17.5 g of saponifiable and 0.19 g non-saponifiable compounds were generated from 50 g of soy-

Table 1. Stimulation of oospore formation in *Phytophthora cactorum* and *P. parasitica* by corn oil and crude soybean lecithin following saponification.

Basal medium supplement	Recovery (%)	Bioassay (µg)	Oospores (No.)	
			P. cactorum	*P. parasitica*
Corn oil				
Untreated	-	100	11,600 ± 1,189	430 ± 53
Non-saponifiable	0.88	10	28,200 ± 2,190	19,630 ± 1,673
Saponifiable	91	100	41,060 ± 4,881	0
Soybean lecithin				
Untreated	-	100	22,780 ± 1,955	2,430 ± 612
Non-saponifiable	0.39	10	31,450 ± 4,847	17,210 ± 1,837
Saponifiable	35	100	15,800 ± 2,683	0
None	-	-	0	0

bean lecithin. Non-saponifiables were also much more active than saponifiables in inducing oospore formation of *P. cactorum*, and only non-saponifiables were stimulatory to *P. parasitica*. Non-saponifiables from corn oil and soybean lecithin were, therefore, selected for further study.

Fractionation of Non-Saponifiables by Florisil Column Chromatography (FCC)

When the non-saponifiables were fractionated on Florisil, a majority of the components was recovered in FCC fraction 3 eluted with 25% ether in hexane (Table 2). About 11 and 6% of the components were in FCC fractions 4 and 2, respectively. Less than 1% was recovered in FCC fraction 1. Among these four fractions, FCC fraction 3 was also the most stimulatory to oospore formation of both *P. cactorum* and *P. parasitica*. FCC fraction 3 from corn oil and soybean lecithin was, therefore, selected for further study. Although FCC fraction 4 was also stimulatory to the oospore formation of both fungi, it was about four to six fold less active than FCC fraction 3 on an equal weight basis. FCC fraction 2 from soybean lecithin was stimulatory to *P. cactorum* and *P. parasitica*, but that from corn oil was stimulatory to *P. cactorum* only. FCC fraction 1 from either corn oil or soybean lecithin was not active.

Fractionation of FCC Fraction 3 by Thin Layer Chromatography (TLC)

Thin layer chromatography separated FCC fraction 3 of both corn oil and soybean lecithin into three major bands with Rf values of 0.37, 0.27 and 0.21 (Table 3). All three bands from corn oil and soybean lecithin were highly stimulatory to oospore formation of *P. cactorum* and *P. parasitica*. Band 3 at Rf 0.21, however, was about three to six fold less stimulatory to *P. parasitica* than either band 1 or 2.

Fractionation of TLC Bands by Digitonin Precipitation

In general, non-digitonides were more stimulatory to oospore formation of *P. cactorum* and *P. parasitica* than digitonides for either corn oil or soybean lecithin (Table 4). Since the non-digitonide fractions from corn oil TLC bands 1 and 2 were among the most active and were obtained in relatively large quantities, they were selected for further characterization.

Separation of Active Components by HPLC

HPLC separation of the non-digitonide fractions from TLC band 1 showed a number of significant peaks (Figure

Table 2. Stimulation of oospore formation in *Phytophthora cactorum* and *P. parasitica* by non-saponifiable components of corn oil and crude soybean lecithin following Florisil column chromatography (FCC).

Basal medium supplement	FCC fraction No.	Eluting solvent	Lipid class eluted	Recovery (%)	Oospores (No. 10 μg^{-1})	
					P. cactorum	*P. parasitica*
Corn oil*	1	Hexane	Hydrocarbons	0.2	0	0
	2	5% Eher in hexane	Sterol esters	5.0	1,430 ± 331	0
	3	25% Ether in hexane	Sterols, triglycerides	83.2	37,600 ± 4,827	10,680 ± 1,588
	4	2% Methanol in ether	Monoglyceride, diglyceride	11.6	8,400 ± 1,140	2,840 ± 1,281
Soybean lecithin*	1	Hexane	Hydrocarbons	0.5	0	0
	2	5% Ether in hexane	Sterol esters	7.6	3,625± 479	178 ± 44
	3	25% Ether in hexane	Sterols, triglycerides	81.2	44,875 ± 4,460	20,750 ± 2,901
	4	2% Methanol in ether	Monoglyceride, diglycerides	10.7	7,260 ± 2,167	5,400 ± 1,816
None	-	-	-	0	0	

*Non-saponifiable compounds from Table 1.

Table 3. Stimulation of oospore formation in *Phytophthora cactorum* and *P. parasitica* by FCC fraction 3 of the non-saponifiables of corn oil and crude soybean lecithin following thin layer chromatography (TLC).

Basal medium supplement	TLC band No.	Rf	Oospores (No. 10 μg^{-1})	
			P. cactorum	*P. parasitica*
Corn oil*	1	0.37	20,400 ± 3,082	19,210 ± 2,772
	2	0.27	24,885 ± 2,187	11,597 ± 2,125
	3	0.21	23,577 ± 2,008	3,349 ± 879
Soybean lecithin*	1	0.37	41,110 ± 3,989	18,060 ± 2,311
	2	0.27	33,898 ± 4,010	19,200 ± 1,776
	3	0.21	22,654 ± 2,246	6,634 ± 1,213
None	-	-	0	0

*FCC fraction 3 of the non-saponifiable component from Table 2.

Table 4. Stimulation of oospore formation in *Phytophthora cactorum* and *P. parasitica* by different TLC fractions of the non-saponifiable components of corn oil and crude soybean lecithin following digitonin precipitation.

Basal medium supplement	Digitonin precipitation	TLC band No.	Rf	Oospores (No. 10 μg^{-1})	
				P. cactorum	*P. parasitica*
Corn oil*	Digitonide	1	0.37	36,250 ± 4,113	18,125 ± 3,400
		2	0.27	46,250 ± 4,291	10,125 ± 1,652
		3	0.21	46,375 ± 3,750	0
	Non-digitonide	1	0.37	50,375 ± 4,767	33,875 ± 3,276
		2	0.27	50,470 ± 3,544	31,750 ± 3,122
		3	0.21	46,875 ± 4,328	625 ± 408
Soybean*	Digitonide	1	0.37	19,250 ± 1,190	7,030 ± 707
		2	0.27	33,875 ± 6,263	7,120 ± 1,081
		3	0.21	43,250 ± 4,349	4,625 ± 1,377
	Non-digitonide	1	0.37	46,500 ± 3,488	13,125 ± 1,887
		2	0.27	58,375 ± 3,544	32,750 ± 3,122
		3	0.21	50,875 ± 3,784	12,050 ± 1,190
None	-	-	-	0	0

*TLC fractions 1, 2 and 3 from Table 3.

1). A total of 13 fractions was collected, each from either a single major peak or two or more adjacent minor peaks. Seven of these 13 fractions were stimulatory to oospore formation of *P. cactorum*, and three were also stimulatory to *P. parasitica* (Figure 1). Since HPLC fractions 5 and 9 were the most stimulatory to oospore formation of both fungi, and were each collected from a single distinct peak, they were selected for further characterization. The HPLC chromatogram of the non-digitonide fraction of TLC band 2 showed several major peaks and a few minor peaks (Figure 2). Among 11 fractions obtained, six were stimulatory to oospore formation of *P. cactorum*, and two were also stimulatory to oospore formation of *P. parasitica*.

Identification of Major Components in HPLC Fractions 5 and 9

The GC chromatograms of active HPLC fractions 5 and 9 showed both major and minor volatile components at the retention times from 4.56 to 24.22 min (Figures 3 and 4). The GC chromatograms of inactive HPLC fractions 4 and 10 showed peaks with retention times from 4.61 to 25.68 min (Figures 5 and 6). The retention times for standard cholesterol, ergosterol, and β-sitosterol were 32.6, 34.5 and 37.2 min, respectively, indicating that sterols were absent in either the active or inactive HPLC fractions tested. The major components identified by GC-MS in HPLC fraction 5 were 3-eicosyne, β-farnesene, tetradecane (2, 6, 10-

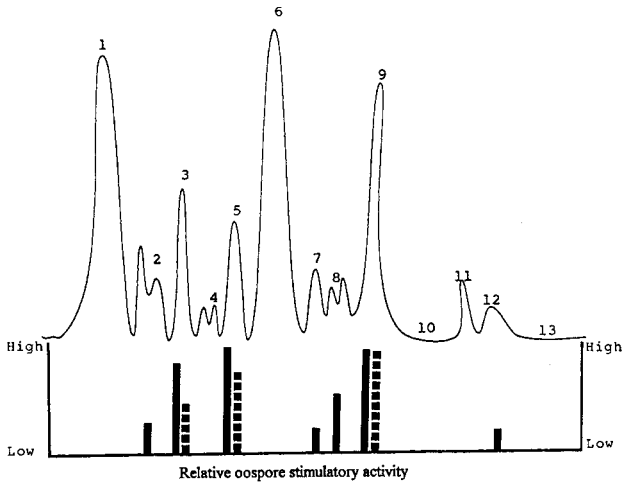

Figure 1. Diagrammatic HPLC chromatogram of the non-digitonide fraction of TLC band 1 from corn oil and oospore stimulatory effect of each fraction on *Phytophthora cactorum* (—) and *P. parasitica* (---). Cutting off fractions were based on peak sequence or shape of chromatogram.

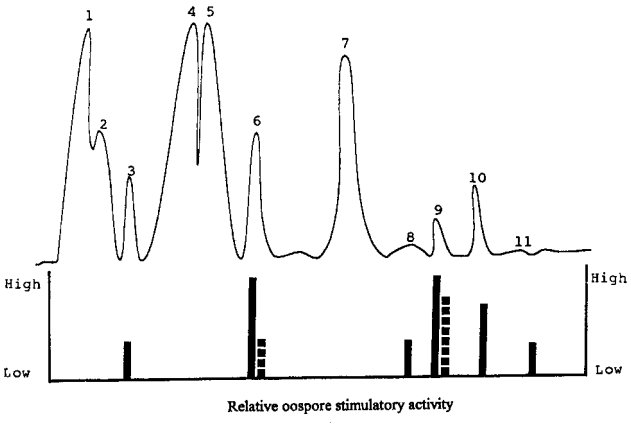

Figure 2. Diagrammatic HPLC chromatogram of the non-digitonide fraction of TLC band 2 from corn oil and oospore stimulatory effect of each fraction on *Phytophthora cactorum* (—) and *P. parasitica* (---). Cutting off fractions were based on peak sequence or shape of chromatogram.

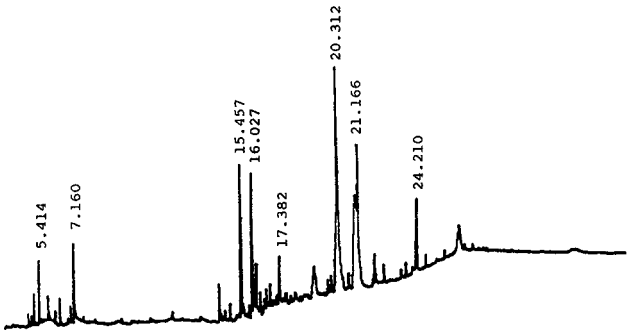

Figure 3. Gas chromatogram of the active HPLC fraction 5 from the non-digitonide fraction of TLC band 1 from corn oil.

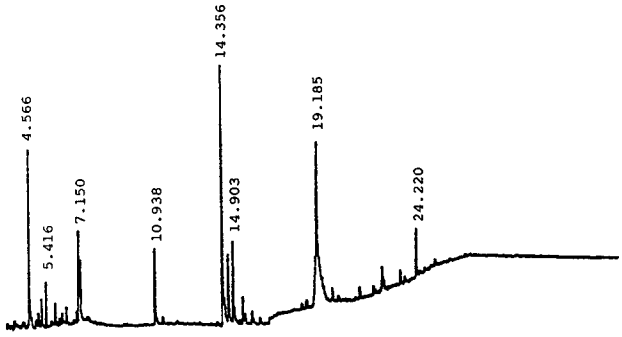

Figure 4. Gas chromatogram of the active HPLC fraction 9 from the non-digitonide fraction of TLC band 1 from corn oil.

trimethyl), and docosane while those in HPLC fraction 9 were hexadecanol (2-methyl), phytol, and pentatriacontane. Since phytol is a common plant natural product, its presence was further confirmed by comparing the GC retention time and mass spectrum with those of a commercial standard (Aldrich), and its oospore stimulatory activity was selected for further study.

Comparison of Oospore Stimulatory Activity of Phytol and Cholesterol

Both phytol (ICN Biochemical, Irvine, CA 92713) and cholestrol were stimulatory to oospore formation of *P. cactorum*, but only phytol was stimulatory to *P. parasitica* (Table 5). The minimum concentrations of phytol and cholesterol for oospore formation of *P. cactorum* were 1 and 10 ng/basal medium disc, respectively. Although the commercial phytol was more active than cholesterol in inducing oogonium formation of *P. cactorum*, most oogonia induced were either immature or with aborted oospores. Based on the amount of normal oospores produced, phytol was much more stimulatory to *P. parasitica* than *P. cactorum* at concentrations ranging from 1 to 100 ng/basal medium disc.

Discussion

Although less than 1% of non-saponifiables was obtained from corn oil and soybean lecithin, this fraction was strongly stimulatory to the sexual reproduction of both *P. cactorum* and *P. parasitica*. The non-saponifiables from lima beans (Harnish et al., 1964), hemp seeds (Harnish et al., 1964), oats (Elliott et al., 1964, Leal et al., 1964), and peas (Leonion and Lilly, 1937; Elliott et al., 1964) have also been reported to be stimulatory to oospore formation of *P. cactorum*. In this study, the 91 and 35% of saponifiables obtained from corn oil and soybean lecithin, respectively, were also found to be stimulatory to oospore formation in *P. cactorum* (Table 1). The main components in the saponifiable fraction are fatty acids (American Oil Chemists Society, 1971; Kates, 1986). Most of the commercial fatty acids tested were stimulatory to the oospore formation of *P. cactorum* after removal of inhibitory substances (Jee and Ko, 1997). Earlier, Leal et al. (1964) and Leonian and

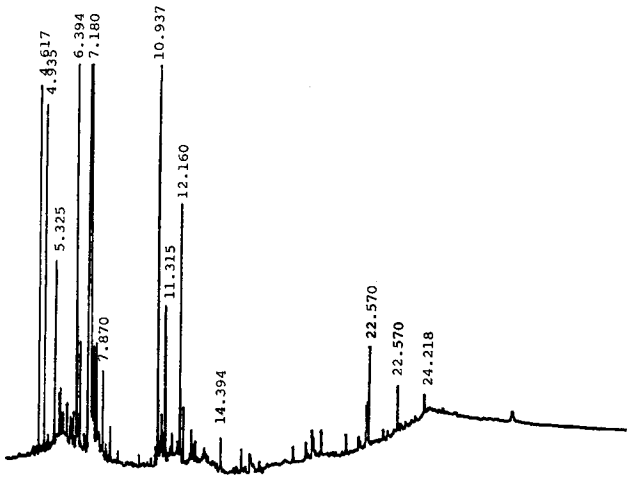

Figure 5. Gas chromatogram of inactive HPLC fraction. 4 from the non-digitonide fraction of TLC band 1 from corn oil.

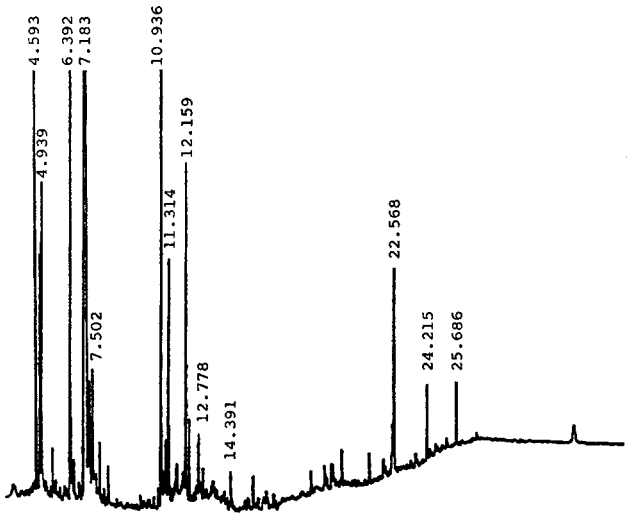

Figure 6. Gas chromatogram of inactive HPLC fraction 10 from the non-digitonide fraction of TLC band 1 from corn oil.

Table 5. Oospore formation in *Phytophthora cactorum* and *P. parasitica* on basal medium disc containing different amounts of phytol or cholesterol.

Amount of supplement (μg)	Oospores of *P. cactorum* (No.)				Oospores of *P. parasitica* (No.)			
	Phytol		Cholesterol		Phytol		Cholesterol	
	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal
10	1,125 (479)*	17,023 (3,240)	6,550 (1,040)	1,987 (750)	2,125 (750)	9,125 (2,322)	0	0
1	1,125 (479)	16,125 (1,701)	4,750 (2,327)	1,125 (866)	2,250 (645)	10,125 (1,314)	0	0
0.1	375 (478)	6,644 (1,080)	950 (114)	400 (353)	4,250 (1,040)	13,300 (2,166)	0	0
0.01	375 (250)	8,125 (1,887)	150 (10)	200 (64)	3,875 (750)	13,500 (1,887)		
0.001	150 (250)	4,875 (1,377)	0	0	4,875 (816)	1,625 (540)	0	0
0.0001	0	0	0	0	0	0	0	0

*Standard deviation.

Lilly (1937) reported that the saponifiables from oats and peas were not stimulatory to oospore formation of *P. cactorum*. However, the possibility of inhibitory substances in their saponifiable fraction can not be ruled out.

Phospholipids are saponifiable (Kates, 1986). Residual phospholipids in the non-saponifiable fraction, if any, would be retained on the Florisil column, and, therefore, FCC fraction 3 should be phospholipids free. Thin-layer chromatography of FCC fraction 3 left the sterol component in TLC band 3. Any possible residual sterols in TLC bands 1 and 2 were eliminated by digitonin precipitation. Therefore, the non-digitonide fraction of TLC bands 1 and 2 should be free from sterols. Band 1 was separated with HPLC into 13 fractions, with most of them containing a single peak. Seven of these were stimulatory to oospore formation in *P. cactorum*. Gas chromatography confirmed the absence of sterols in the active HPLC fractions 5 and 9. These results support the conclusion that there are compounds in corn oil which are not sterols, phospholipids or fatty acids, but are stimulatory to sexual reproduction in species of *Phytophthora.*

Phytol detected in HPLC fractions 5 and 9 from corn oil was stimulatory to oospore formation of both *P. cactorum* and *P. parasitica*. Since phytol is a common constituent of plants (Robinson, 1991), it may be an important factor in the survival of this group of fungi in nature.

Most of the major components in active HPLC fractions 5 and 9 were aliphatic hydrocarbons. Whether any of these aliphatic hydrocarbons is stimulatory to sexual reproduction of pythiaceous fungi remains to be investigated.

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對疫病菌 *Phytophthora cactorum* 及 *P. parasitica* 有性生殖具有刺激作用之植物化合物之特性分析

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在添加了玉米油或粗製大豆卵磷脂之基礎培養基中 *Phytophthora cactorum* 及 *P. parasitica* 能產生卵孢子。二種添加物中之非皂化成份對此二疫病菌產生卵孢子均有刺激效用，但是其皂化成份則只能刺激 *P. cactorum* 產生卵孢子。添加物之非皂化成份經以 Florisil 管柱層析法（column chromatography）純化，由 25% 乙醚／己烷溶析出之成份對刺激卵孢子之形成最有效。此成份之物質經薄層層析法（TLC）分析後形成 3 個色帶 (bands)，其中的二個 bands 含非毛地黃皂甘 (non-digitonide) 物質，經高效率液相層析法 (HPLC) 純化後，分離出其有效成份，再以氣相層析-質譜儀（GC-MS）做化學鑑定。鑑定出之化合物有 3-eicosyne, β-farnesene, tetradecane (2, 6, 10-trimethy), docosane, hexadecanol (2-methyl), phytol 及 pentatriacontane。市售之植醇 (phytol) 經測試後發現對此二疫病菌株卵孢子之形成具有很強之刺激性，即使在 1 ng／基礎培養基圓盤之濃度下仍具有很高的刺激性。

關鍵詞：玉米油；大豆卵磷脂；植醇；有性生殖；*Phytophthora cactorum*；*P. parasitica*。