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STEROL CONTENT OF MATURE CEREAL STRAW: POSSIBLE ROLE IN OOSPORE FORMATION IN *PHYTOPHTHORA*

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Key Word Index—*Avena fatua*; *Hordeum vulgare*; *Triticum aestivum*; straw; *Phytophthora porri*; oospores; phytosterols; sitosterol; stigmasterol; campesterol; cholesterol.

Abstract—The main sterols of mature barley, wheat and wild oat straw were sitosterol, stigmasterol and campesterol at approximately 60, 18 and 15%, respectively. The sterols were distributed in the four sterol classes free sterols, steryl esters, steryl glucosides and acylated steryl glucosides at about 53, 25, 17 and 8%. The total sterol content for barley, wheat and wild oat straw was 668, 391 and 490 μg/g fr. wt, respectively. A species of *Phytophthora* causing serious root rot of carrots in Alberta, recently identified as *P. porri*, was induced to form oospores following long-term incubation with cereal straw sections. Therefore, under field conditions, where these cereal straws are abundant, they may serve as a natural source of sterols which are required for oospore production and thus be of significance in accounting for the survival of this fungus in the field.

INTRODUCTION

Fungi of the genera *Pythium* and *Phytophthora* are unable to synthesize squalene oxide and thus do not synthesize sterols [1]. However, sterols are required in the growth media of both homothallic and heterothallic species of *Phytophthora* for oospore formation and the sterols common to higher plants are the most effective [2, 3]. The oospore is a sexually produced, environmentally resistant spore providing long-term survival and its morphology is widely used in species identification [2, 3].

A previously undescribed species of *Phytophthora*, causing serious root rot on carrots (*Daucus carota* L.) grown under irrigation in Alberta, was isolated [4] and later identified as *Phytophthora porri* Foister [5]. Initial attempts to stimulate oospore formation in these isolates using mature wheat straw partially embedded in agar, as described by De Bruyn [6], were successful. Therefore this study was undertaken, in part, to determine whether straw from several species used in such culture would induce oospore formation in isolates of *Phytophthora porri*. But, the major objective was to perform a detailed analysis of the sterols present in mature straw.

RESULTS AND DISCUSSION

The oospores were not found until 55 weeks in culture and were later observed on all straw cultures tested with isolates of *Phytophthora porri*. In addition to the wheat, barley and wild oats samples, which were used for sterol analysis, rye (*Secale cereale* L.) and perennial fescue (*Festuca* sp.) stem sections also stimulated oospore formation.

The abundance of various sterol classes in the mature straw segments is given in Table 1. The free sterol fractions contained the highest sterol content for all the straw types analysed, ranging from 42 to 64% and the acylated sterol glucoside fraction the lowest, 5–13% (Table 1). Barley straw contained the highest overall sterol content at 668 μg/g (Table 2). Sitosterol was the major sterol in all lipid fractions, comprising 50–67% of the sterols present (Tables 2 and 3). The values for the wheat straw are in partial agreement with studies by Izzo *et al.* [7] and Willemot [8] on wheat leaf samples, except they report a very low stigmasterol level (3–6%) and a higher campesterol level, up to 29% compared to about 19 and 18%, respectively, for wheat straw (Tables 2 and 3). The higher cholesterol and lower campesterol content in *Avena fatua* compared to the values for wheat and barley (Tables 2 and 3) has also been reported for cultivated oats, *Avena sativa* L. [9, 10]. The sterols are typical of those found in higher plants [11]. The overall sterol contents of 390–670 μg/g fr. wt (Table 2) are approximately 60% of the estimates reported for green wheat leaves at 245 μg/g fr. wt [8] and 1497 μg/g dry wt for oat roots [12] when adjustments are made for moisture content (root samples approximately 20% fr. wt [12] and mature cereal straw contains approximately 10% moisture [13]).

Sterols in plants are considered mainly membrane components, although some evidence suggests that they may serve as storage and transport molecules [11, 14, 15]. During senescence [16] and water deficit stress [12], they have been shown to be the more stable components of the

Table 1. Relative amounts of the sterol classes found in mature straw of wheat, barley and wild oats

	Sterol class (%) (mean ± s.e. of 3 samples)			
	SE*	FS	ASG	SG
Wheat	20.2 ± 1.8	64.1 ± 8.7	5.1 ± 4.2	10.5 ± 2.6
Barley	40.1 ± 6.6	51.6 ± 5.7	7.2 ± 0.5	12.2 ± 1.1
Wild oats	15.1 ± 1.6	42.0 ± 1.7	13.6 ± 0.8	29.1 ± 1.0

*SE: steryl esters; FS: free sterols; ASG: acylated steryl glucosides; SG: steryl glucosides.

Table 2. Total sterols and relative amounts of the main sterols in mature straw of wheat, barley and wild oats (means ± s.e. for 5 samples)

	Sterols (%)				Total sterols (μg/g fr. wt)
	Cholesterol	Campesterol	Stigmasterol	Sitosterol	
Wheat	3.2 ± 0.4	17.9 ± 0.2	19.4 ± 0.2	59.5 ± 0.5	391 ± 32
Barley	1.9 ± 0.1	18.2 ± 0.2	15.9 ± 0.6	65.2 ± 0.5	668 ± 34
Wild oats	7.3 ± 0.3	10.8 ± 0.1	19.6 ± 0.2	62.1 ± 0.4	490 ± 18

Table 3. Main sterol components of steryl ester (SE), free sterol (FS), acylated steryl glucoside (ASG) and steryl glucoside (SG) fractions of mature wheat, barley and wild oats straw. Mean ± s.e. of 3 samples

		Sterol composition (%)			
	Fraction	Cholesterol	Campesterol	Stigmasterol	Sitosterol
Wheat	SE	6.0 ± 0.1	17.0 ± 0.7	9.6 ± 0.7	67.3 ± 1.6
	FS	3.5 ± 0.1	18.2 ± 0.2	23.5 ± 0.1	54.7 ± 0.2
	ASG	2.8 ± 1.8	20.4 ± 2.2	23.3 ± 2.0	54.0 ± 1.9
	SG	7.4 ± 3.0	17.8 ± 0.3	19.8 ± 0.7	54.7 ± 2.3
Barley	SE	3.5 ± 0.4	18.8 ± 0.2	12.3 ± 1.0	65.4 ± 1.3
	FS	2.1 ± 0.2	17.9 ± 0.2	18.6 ± 0.9	61.3 ± 1.1
	ASG	0.7 ± 0.5	17.6 ± 0.3	14.2 ± 0.9	67.3 ± 1.2
	SG	0.8 ± 0.5	18.6 ± 0.5	13.0 ± 1.2	67.6 ± 1.5
Wild oats	SE	10.6 ± 0.8	10.3 ± 0.2	28.0 ± 1.2	50.8 ± 2.8
	FS	9.3 ± 0.7	9.8 ± 0.4	27.9 ± 0.5	52.2 ± 1.5
	ASG	7.4 ± 0.6	11.1 ± 0.4	16.5 ± 0.7	65.0 ± 0.9
	SG	6.0 ± 0.4	11.5 ± 0.1	15.6 ± 0.3	66.9 ± 0.3

membrane system. A substantive loss of the acyl lipids results in a sharp increase in the sterol/acyl lipid ratio [12, 16]. Labelling studies have also suggested a slow turnover of the sterol moiety in plants [17].

Nes *et al.* [18] reported that the optimum sterol concentration required for oospore formation is 10–20 mg/l., with cultures containing 1 mg/l. producing 10% of the number of oospores formed at the optimum. We estimate the straw inserts might give a sterol content of approximately 3 mg/l. and this was localized near the inoculum. Corn meal agar used for a portion of these studies was analysed to have a sterol content of 1 μg/g giving a final sterol content in the medium of 40–60 μg/l., which is far below the optimum concentration for oospore formation, which was not observed in the controls.

The exact role of the required sterols in oospore development in *Phytophthora* is not known. A possible hormonal role is suggested from studies on another oomycete, *Achlya*, where the hormones controlling oospore formation, antheridiol and oogoniol, were shown to be directly derived from the sterol fucosterol [19]. However, unlike *Phytophthora*, *Achlya* supports *de novo* synthesis of sterols. A hormonal nature in the stimulation of oospore formation in *Phytophthora* is suggested from the mating studies of Ko [20], where isolates on agar were separated by polycarbonate filters. Homothallic strains could induce oospore formation in heterothallic strains when paired; opposite mating types of heterothallic strains were also induced to form oospores when paired [20, 21]. The species used in our study was also induced to form oospores in pairing studies [5]. Hormones similar to oogoniol and antheridiol produced by *Achlya* have not been detected in *Phytophthora* [2, 3] nor was the addition of antheridiol to the medium effective in inducing oospore formation [22]. *Phytophthora* have specific enzyme systems to esterify, glycosolate and metabolize the supplemented sterols [2, 3], and Elliott [2] suggested some of the polar products produced include precursors of hormones controlling oospore formation. Several sterols also promote vegetative growth which may be due to their ability to serve as membrane components [2, 3]. However, a comparison of the relative activities of several sterols on oospore formation showed that the two separate developmental stages, oogonium and antheridium formation and subsequent oospore development, have different sterol requirements [2, 3, 23]. Elliott [2] suggested that these differing requirements are compatible with hormonal types of action.

The *Phytophthora* species used in this study, recently identified as *P. porri* [5], is considered a homothallic species [24, 25]. The aging requirement for oospore formation found in this study and also reported by Ho [5] for this species is more typical of single isolates of heterothallic species grown in medium with adequate sterol sources [26–28]. However, some species classified as homothallic have been shown to require aging and/or stimulation by other homothallic species or other heterothallic mating types for oospore formation [24]. In

addition to sterols, mating types and aging, environmental factors [24] may play a role in oospore formation in some cultures. At present, the very long incubation period required for oospore formation in *Phytophthora porri* makes elucidation of the exact role of sterols difficult.

EXPERIMENTAL

Phytophthora *culture.* Three isolates of *Phytophthora* from carrots affected with rubbery brown rot, initially described by Stelfox and Henry [4], were used to inoculate sterile tubes containing sections of mature cereal straw partially embedded in water or corn meal agar. The inoculum consisted of approximately 1 cm^3 pieces of agar bearing mycelium of the carrot *Phytophthora* added to each tube on the surface of the agar and in contact with the partially embedded straw. The tubes were capped and incubated in a growth cabinet held at 15°.

Plant material. The straw for all the species used in the study was from the University of Alberta farm at Edmonton. The three species used in the analysis of the sterols were *Hordeum vulgare* (barley, cv. Conquest), *Triticum aestivum* (wheat, cv. Neepawa) and *Avena fatua* (wild oat).

Chemicals. Cholesterol, campesterol, stigmasterol, coprostanol, cholestanol, desmosterol, lanosterol, fucosterol, ergosterol, esterified steryl glucoside, steryl glucoside and plant sterol mixture were purchased from Supelco. Cholestane and sitosterol were obtained from Aldrich and cholesterol oleate was obtained from Sigma.

Isolation of sterols. Straw samples (2–5 g) consisting of 15 cm stem sections taken immediately below the spike or panicle were ground to a fine powder in liquid nitrogen and then homogenized in $CHCl_3$–MeOH (2:1) for 2 min using a Polytron. The resulting mixture was then refluxed for 1 hr and filtered through Whatman No.1 filter paper. The non-lipid contaminants were removed with Sephadex G-25 [29] which involved the addition of 3 g of Sephadex to the filtrate. To facilitate swelling, 3 ml H_2O was added. The sterols were eluted with 100 ml $CHCl_3$ followed by 70 ml $CHCl_3$–MeOH (2:1). Further elution of the Sephadex with $CHCl_3$–MeOH (1:1) eluted a yellow pigment, which when monitored by TLC using $CHCl_3$–MeOH (8:1) solvent systems remained at the origin and was thought to be flavonoid [29]. Sterols were separated by TLC on glass plates (20 × 20 cm) coated with a 250 μm layer of silica gel HR (Merck) using $CHCl_3$–MeOH (8:1) as the solvent system. Samples were applied as horizontal bands. A mixture of sterol standards was applied as a single spot on one edge of the same plate. Sterol zones were detected by spraying the sterol standards lane with Rhodamine 6G (Allied Chemical), with the sample portion of the plate covered and outlined under UV. Bands corresponding to steryl ester, free sterol, steryl glucoside and acylated steryl glucoside were collected into 15 ml screw-capped tubes (with Teflon liners). Steryl esters and free sterols were saponified with 6% KOH in MeOH–H_2O (9:1) for 1 hr at 90° [30] and the sterols were extracted in *n*-hexane. Acylated steryl glucosides and steryl glucosides and total sterol fractions were hydrolysed for 4 hr at 90° in 5 ml 0.5% H_2SO_4 in MeOH and then saponified with the addition of 3 ml 10% KOH–MeOH for 30 min [31]. The sterols were extracted in *n*-hexane, concentrated to dryness and resuspended in $CHCl_3$–MeOH (2:1). Sterols were further purified by TLC on silica gel HR using $CHCl_3$–MeOH (8:1) as the solvent system. The free sterol zone was eluted with $CHCl_3$–MeOH (2:1), concentrated to dryness and resuspended in EtOAc. Cholestane was added as an internal standard and the free sterols were separated on an Aerograph Model 200 gas chromatograph equipped with a flame ionization detector. A coiled stainless-steel column (1.8 m × 3 mm) packed with 5% OV-101 on Anachrome AS 80/100 mesh (Analabs) was used with helium as the carrier gas [32]. Individual sterols were identified by comparison with authentic standards and quantified by peak area relative to cholestane.

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