Inhibition of *Aphanomyces euteiches* f. sp. *pisi* by Volatiles Produced by Hydrolysis of *Brassica napus* Seed Meal

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ABSTRACT

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Seed meal from *Brassica napus* (rapeseed) produced volatile fungitoxic compounds potentially of value in the control of *Aphanomyces* root rot of pea. Hyphal growth, germination of encysted zoospores, and oospore survival and inoculum potential, were determined in the presence of volatiles produced from *B. napus* seed meal. Volatile compounds from *B. napus* meal completely suppressed mycelial growth and germination of encysted zoospores on agar. In growth chamber bioassays, pea (*Pisum sativum*) seed inoculated with zoospore suspensions and incubated 24 h in the presence of volatiles from rapeseed meal had 50% lower root rot disease severity than in the absence of meal. Volatile compounds passing through soil also significantly decreased survival and inoculum potential of oospores. Gas chromatographic analysis of rapeseed tissues and the volatile compounds evolved from tissues showed that substrate glucosinolates were hydrolyzed enzymatically to produce mainly isothiocyanates. Non-autoclaved rapeseed meal produced significantly higher levels of volatile compounds than did autoclaved meal. Also, volatile compounds produced from autoclaved meal were dominated by nitriles, whereas isothiocyanates were more common volatile products from non-autoclaved meal. Our results indicate that *B. napus* allelochemicals responsible for toxic effects toward *A. euteiches* f. sp. *pisi* are enzymatic hydrolysis products of glucosinolates.

Aphanomyces root rot of pea is one of the most economically important diseases of pea (*Pisum sativum* L.) (26,31). The fungal pathogen *Aphanomyces euteiches* Drechs. f. sp. *pisi* W. F. Pfender & D. J. Hagedorn infects germinating seeds, epicotyls at the early seedling stage of growth, and pea roots at any growth stage (31). Oospores, the dormant resting stage of the pathogen, may survive in soil for several years (29). Different plants can serve as hosts, making possible a continued renewal of the fungal population even in the absence of a pea crop (7), thus making crop rotation less effective as a disease control strategy. Effective fungicides to control *Aphanomyces* root rot are not currently available. However, several studies have shown that soil amendment with cruciferous plants can reduce levels of certain soil-borne plant pathogens, including *A. euteiches* (4,12,16,17,21,23,27). Rapeseed (*Brassica napus* L.), like other plants in the family Cruciferae, contains compounds called glucosinolates (14,32,33). Glucosinolates are found in all parts of the rapeseed plant, but the highest concentrations

are in the seed (14). Glucosinolates are degraded enzymatically to yield a variety of important compounds including isothiocyanates, nitriles, thiocyanates, oxazolidinethiones, and epithionitriles (3,11,14), all of which may exhibit toxicity to microorganisms (5,6,9,10,15,16,28,33). The enzyme responsible for glucosinolate degradation is thioglucoside glucosylhydrolase (EC 3.2.3.1), also called myrosinase (18). The enzyme is separated from the glucosinolates in intact plant tissues, but is released when the tissues are crushed or when autolysis within the plant occurs (14,32). Glucosinolates are stable in dry plant material and defatted seed meal (33).

The effectiveness of cruciferous plant amendments against *Aphanomyces* root rot was demonstrated by Lewis and Papavizas (16) and Chan and Close in the greenhouse (4), and by Muehlchen et al. (21) in the field. Lewis and Papavizas showed that volatiles from the decomposition of air-dried leaf and stem tissue of cabbage in soil adversely affected mycelial growth and development of oospores of *A. euteiches* (16).

Although the evidence suggests a relationship between volatile glucosinolate hydrolysis products and suppression of *A. euteiches*, it has not been specifically demonstrated that glucosinolate degradation products are responsible for toxic effects toward the pathogen. This research evaluated the effect of volatile compounds from hydrated rapeseed meal on growth stages

of *A. euteiches* f. sp. *pisi*, including hyphal growth, encysted zoospore germination, and survival and inoculum potential of oospores. Chemical characterization of rapeseed tissues and the resulting volatile products was performed to establish whether there is a clear link between glucosinolate-derived compounds and bioassay results.

MATERIALS AND METHODS

Bioassay components. Our isolate of *A. euteiches* f. sp. *pisi* was obtained from W. J. Kaiser (USDA-ARS, Pullman, WA) and maintained on potato dextrose agar (Difco Laboratories, Detroit, MI) at 4°C.

Palouse-Latahco silt loam soil was obtained from the University of Idaho Parker Farm near Moscow, ID. The soil contained 20% sand, 20% clay, and 60% silt (University of Idaho Analytical Services Laboratory). Soil pH in a soil/water (2:1) paste was 5.9. Air-dried soil was sieved (0.85 mm mesh), autoclaved (1 h at 121°C), and autoclaved again after 24 h for 30 min. Silica sand (Lane Mt. 20/30, Silica Co., Valley, WA) with a pH of 6.0 was used in those experiments noted.

Defatted seed meal of *B. napus* cv. Dwarf Essex was used as the glucosinolate-containing tissue for these experiments. This tissue was obtained after the industrial oil was removed with a pilot scale press at the University of Idaho (25) and was used raw (without autoclaving, designated "Rs") or after it was autoclaved for 20 min at 121°C ("ARs"). Glucosinolate concentrations in both types of meal were determined with the procedure described by Brown et al. (3).

Analysis of volatiles from rapeseed meal. Volatile compounds were produced by first placing 100-mg samples of Rs or ARs meal into 30-ml coarse fritted disk funnels (40 to 60- μ m pore size; Corning 36060-30C). The meal in each flask was moistened with 100 μ l of 0.1 M phosphate buffer (pH 6.5). Volatile compounds were collected by pulling air (25 ml/min) through the tissue and then a trap consisting of 10 mg of Tenax TA (60/80 mesh; Supelco, Bellefonte, PA) in 16-cm $\times$ 0.4-cm-inner-diameter glass tubes, for periods ranging from 30 min to 24 h. The Tenax trap was dried with a stream of nitrogen and inserted into a purge and trap injector (Chrompack International, Middelburg, The Netherlands). Compounds were injected under the following conditions: 2

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min pre-cooling, 5 min desorption from the Tenax trap at 250°C, and 3 min injection from the cryotrap at 200°C. Gas chromatographic analysis of the injected sample was performed as previously described (1,3).

Mycelial growth in the presence of volatile allelochemicals. The fungus was allowed to grow on half-strength Difco corn meal agar for 3 to 5 days at 25°C. An agar disk (5 mm diameter) was cut from the colony edge and transferred to the center of a new corn meal agar plate, which was subsequently placed in a large (15 cm diameter) glass petri dish. An aluminum weighing dish containing either 5 g of rapeseed meal (either Rs or ARs) or 10 ml of distilled water was also placed in the glass dish. Distilled water (10 ml) was added to the rapeseed meal and the glass petri plates were quickly covered and sealed with plastic laboratory film to prevent the escape of volatile compounds. Dishes were incubated at 25°C and radial colony growth was measured daily for 8 days. There were three replicates per treatment and the experiment was repeated once.

Encysted zoospore germination and inoculum potential in the presence of volatile compounds from rapeseed meal. Zoospores were produced using a modification of the method of Mitchell and Yang (20). The fungus was grown for 3 days at 25°C in an aqueous solution of 0.5% dextrose and 2% peptone, in plastic petri dishes. To induce zoospore formation, mycelium was rinsed twice, for approximately 30 s each time, with a solution containing 1.75 mM CaCl₂, 1 mM KCl, and 1 mM MgSO₄·7H₂O, then subsequently rinsed again with the solution at 2- and 4-h intervals. Zoospores were released by chilling the cultures for 20 min at 7°C, 20 h after the final rinse. Between rinses, and after the final rinse, cultures were kept at 25°C. Zoospores were enumerated with a hemacytometer, and zoospore concentration was adjusted to approximately 5 × 10²/ml.

Sterile microscope slides were dipped in melted corn meal agar to obtain a thin agar layer on the slide surface, then placed in glass petri dishes (9 cm diameter) lined with moistened filter paper. A 100-μl sample of the zoospore suspension was gently spread on each slide. An uncovered watch glass containing either 1.2 ml of sterile water (control) or 600 mg of rapeseed meal (Rs or ARs) was placed in each dish. After adding water (1.2 ml) to the meal, dishes were covered and sealed with plastic film. After 20 h of incubation at 25°C, all of the encysted zoospores on each slide were counted microscopically at a magnification of ×100, and the percentage of germination was determined. There were three replicates per treatment and the experiment was repeated twice.

In a separate experiment, peas (cv. Columbia) were placed in sterile water for 24 h. Then, 30 imbibed seeds were immersed in a zoospore suspension (10² to 10³ zoo-

spores/ml) for 10 min at 20°C. Peas then were removed and placed on paper coffee filters (10 peas per filter, with wet filter paper underneath). Coffee filters with peas were placed in glass jars (480 cm³) containing 50 ml of distilled water to maintain humidity, plus an aluminum weighing dish containing 10 g of rapeseed meal (Rs or ARs) or 10 ml of water. Water (20 ml) was added to the meal and the jars were sealed with plastic screw caps and plastic film. There were three replicates per treatment. After 24 h at 22 to 25°C, the peas were removed, planted in sand in plastic seedling tubes (2.5 × 20.5 cm, Ray Leach Cone-Tainer, Canby, OR), and periodically watered. After 18 days at 20°C, seedlings were removed, the roots were washed, and symptom severity was rated on a 0 to 4 rating scale for *Aphanomyces* root rot (0 = plant healthy; 1 = root slightly discolored; 2 = root extensively discolored but not shrunken; 3 = root extensively discolored and shrunken; and 4 = root partially or completely rotted or plant dead) (24). Wet weights of the whole plant and the root system were obtained for each plant. The experiment was repeated once.

Oospore survival and inoculum potential in the presence of volatile compounds. Oospores of *A. euteiches* f. sp. *pisi* were produced in V8 broth culture (8), and used within 1 day. Silt loam soil was amended with oospores at a density of 500 oospores g⁻¹ of air-dry, autoclaved Palouse-Latahco silt loam soil (oospores were enumerated with a hemacytometer), and soil moisture was adjusted to -80 kPa (21%) according to a predetermined soil moisture curve. Ten grams of rapeseed meal (Rs or ARs) was placed on the bottom of a glass jar (480 cm³) and 15 ml of water was added. The rapeseed meal was immediately covered with fiberglass cloth after which 121 g of moistened oospore/soil was added. Jars without meal served as controls. The jars were sealed with plastic screw caps and kept 10 days at 22 to 25°C. There were three replicates per treatment and the experiment was repeated twice. After 10 days, the soil was removed from the jars for enumeration of oospores and for bioassay. To estimate the number of oospores in the soil, microscope slides

were first coated with a thin layer of water agar, then placed in a moist chamber lined with wet filter paper. One gram of soil from each sample was suspended in 1 ml of Calcofluor fluorescent brightener (Sigma, St. Louis, MO) (280 ppm) and incubated for 2 h at 25°C in the dark. A 4-ml volume of sterile, distilled water was added to this mixture and a 1-ml sample was dispersed on the agar-coated slide. Three slides were prepared for each soil sample. Oospores were counted at a magnification of ×100, using epifluorescence.

The inoculum potential of oospores from the above treatments was assayed in plastic seedling tubes (2.5 cm diameter × 12 cm length). The bottoms of tubes were plugged with sterile cotton, then sterile sand was added to a depth of 4 cm, followed by a 10-ml volume (2 cm deep) of soil from one of the jars. This was overlaid with 3 mm of sand, on top of which a single pea seed was laid and then covered with an additional 2 cm of sand. After 18 days at 20°C, seedlings were removed from the tubes and the roots were washed and rated on the 0 to 4 disease rating scale. Wet weights of whole plants and roots were obtained for each plant. There were 25 plants per treatment and the experiment was repeated once.

In a second experiment to evaluate the effect of volatile compounds from rapeseed meal, soil was infested with oospores at a level of 500 oospores per g. Noninfested soil served as a control. Soil samples (24 g each) were placed in uncovered plastic petri dishes, and four dishes were placed in a covered plastic container (20 × 20 × 9 cm) along with an aluminum weighing dish containing either 30 ml of water or 20 g of rapeseed meal (Rs or ARs) moistened with 30 ml of water. There were three replicate boxes per treatment. After a 7-day incubation at 22 to 25°C the soil was removed, oospores were enumerated, and seedling bioassays were performed as described above.

RESULTS

Glucosinolates and volatile compounds. Both intact and autoclaved meals contained similar concentrations of the measured glucosinolates (Table 1). Both

Table 1. Content of glucosinolates in intact (raw) and autoclaved rapeseed meal

Glucosinolate	Glucosinolate content (μmol per g of meal)	
	Intact rapeseed meal	Autoclaved rapeseed meal
Allyl	0.3	0.2
3-Butenyl	37.9	36.4
4-Pentenyl	12.8	12.5
2-Hydroxy-3-butenyl	83.2	80.4
2-OH-4-Pentenyl	6.7	6.5
4-Methylthiobutyl	0.9	1.0
Phenethyl	2.5	1.9
5-Methylthiopentyl	0.7	0.9
Indol-3-ylmethyl	0.2	0.0
4-Hydroxyindol-3-ylmethyl	5.1	4.0
Benzyl	6.3	5.0

types of rapeseed meal contained large amounts of 2-hydroxy-3-butenyl, 3-butenyl, 4-pentenyl glucosinolate, and seven other glucosinolates in smaller quantity.

Relative concentrations of volatile compounds in the gas phase for analyzed samples of rapeseed meal were determined, and the sum of all peaks occurring in each chromatogram was used as a reference for the relative concentration of volatiles in the gas phase. Intact rapeseed meal produced a higher concentration of volatile compounds than did autoclaved meal. Two hours after hydrating, autoclaved meal produced approximately 36% as much volatile compounds as did non-autoclaved meal. Concentrations of volatiles decreased over a 24-h period for both intact and autoclaved meals, during which time it was possible to recognize more than 50 compounds in the gas phase. Significant differences in profiles and amounts of volatile compounds from hydrated autoclaved and intact rapeseed meal were recorded (Fig. 1). Volatile compounds produced from autoclaved rapeseed meal were dominated by nitriles, whereas isothiocyanates were more common volatile products from intact rapeseed meal.

Mycelial growth in the presence of volatile compounds. Volatile compounds released from hydrated rapeseed meal completely prevented mycelial growth of *A. euteiches* f. sp. *pisi* (Fig. 2). Mycelial

growth in the presence of volatiles from autoclaved meal was not different from the control treatment (analysis of variance, $P \geq 0.05$).

Encysted zoospore germination and inoculum potential in the presence of volatile compounds. Volatiles from intact rapeseed meal completely inhibited germination of encysted zoospores (data not shown). Volatiles released from autoclaved meal significantly reduced the germination of encysted zoospores ($P < 0.05$) from a mean of 128 per slide in the control to 84 per slide for the autoclaved meal treatment (i.e., a 34% reduction). The treatment by experiment interaction was not significant ($P \geq 0.05$) for evaluation of zoospore inoculum potential; therefore, data were pooled. Volatile compounds released from both types of rapeseed meal (intact and autoclaved) significantly ($P < 0.05$) reduced the severity of root rot of plants grown from seeds inoculated with zoospores (Table 2). Root rot ratings of treatments with intact meal were significantly lower than ratings of treatments with autoclaved meal ($P < 0.05$). In the treatment with intact rapeseed meal, plants grown from seed inoculated with zoospores of *A. euteiches* f. sp. *pisi* had weights similar to those observed in the corresponding treatment without the pathogen (Table 2). However, plant weights were lower in treatments with the pathogen, when auto-

claved meal was used (Table 2). Plants from seeds not inoculated with the pathogen but exposed to volatiles from either type of rapeseed meal had significantly ($P < 0.05$) lower fresh weights than control plants; both intact and autoclaved meal reduced average plant weight by approximately 10%. Addition of *A. euteiches* f. sp. *pisi* zoospores to the intact meal treatment resulted in no further decrease in mean plant weight; however, addition of zoospores to the autoclaved meal treatment resulted in plant weights similar to those observed with the pathogen alone (Table 2).

Fig. 2. Colony diameter of *Aphanomyces euteiches* f. sp. *pisi* in the presence of volatiles from hydrated rapeseed meal. Δ = Rs, rapeseed meal; $\square$ = Ars, autoclaved rapeseed meal; $\circ$ = water control.

Autoclaved

Intact

Fig. 1. Gas chromatographic-mass spectrometric analysis of volatile compounds collected from autoclaved and intact rapeseed meal over 24 h after hydration. Identified compounds: 1 = hydrogen sulfide; 2 = benzene; 3 = butenenitrile; 4 = pentenenitrile; 5 = hexenenitrile; 6 = butenyl isothiocyanate; 7 = nitropentene; 8 = 5-methylthiopentanenitrile; 9 = 2-phenylethyl nitrile; 10 = benzenepropanenitrile; 11 = 1,3 benzenedicarboxylic acid; 12 = 3-methyl butanal; 13 = hexanal; 14 = isopropyl isothiocyanate; 15 = 3(E)-hexene; 16 = allyl isothiocyanate; 17 = 2-methylpropyl isothiocyanate; 18 = pentenyl isothiocyanate.

Table 2. Effect of volatile compounds from hydrated rapeseed meal on zoospore inoculum potential of *Aphanomyces euteiches* f. sp. *pisi*

Treatment ^x	Aphanomyces rating ^y	Plant weight (g) ^z
Without pathogen		
No meal	...	1.54 a
Rs	...	1.38 b
Ars	...	1.39 b
Pathogen present		
No meal	1.6 a	1.25 c
Rs	0.8 c	1.37 b
ARs	1.2 b	1.25 c

^x Peas were either exposed to a zoospore suspension (5×10^2 zoospores/ml) or not, then incubated 24 h in the absence of rapeseed meal, or in the presence of non-autoclaved meal (Rs) or autoclaved meal (ARs). All treatments then were planted in sterile sand and allowed to grow for 18 days.

^y Seedlings were rated on a 0 to 4 rating scale for *Aphanomyces* root rot: 0 = plant healthy; 1 = root slightly discolored; 2 = root extensively discolored but not shrunk; 3 = root extensively discolored and shrunk; and 4 = root partially or completely rotted or plant dead (24). Means ($N = 60$) in the same column followed by the same letter are not statistically different (least significant difference [LSD]; $P \geq 0.05$). Plants in the noninfested soil had a disease rating of zero.

^z Wet weight of the plant. Means ($N = 60$) in the same column followed by the same letter are not statistically different (LSD; $P \geq 0.05$).

Oospore survival and inoculum potential in the presence of volatile compounds. The treatment by experiment interaction was not significant ($P \geq 0.05$) and data from both repetitions of the experiment were pooled. Numbers of oospores recovered from soil differed significantly ($P < 0.05$) among treatments. The highest numbers of oospores (285 oospores per g) were observed from the soil that was not exposed to either intact or autoclaved meal; significantly ($P < 0.05$) lower numbers (223 oospores per g) were observed from the soil exposed to volatiles from autoclaved meal, and the lowest numbers (154 oospores per g) were observed from soil exposed to intact rapeseed meal. However, due to the difficulty of inducing oospores of *A. euteiches* f. sp. *pisi* to germinate, it is not known what proportion of the recovered oospores remained viable.

When volatile compounds from either intact rapeseed meal or autoclaved meal were allowed to pass through oospore-infested soil, subsequent root rot of pea plants was strongly suppressed ($P < 0.05$) (Table 3). There was no significant difference ($P > 0.05$) between the intact and autoclaved treatments. Plant and root weights from noninfested soil or from infested soil exposed to volatiles from either intact or autoclaved meal were significantly greater ($P < 0.05$) than from infested soil that was not exposed to these volatile compounds. Differences in root and plant weights between intact and autoclaved rapeseed meal were not significant ($P > 0.05$). Root rot ratings for infested soils exposed to volatiles from either intact or autoclaved meal remained significantly lower than the infested control, and were not significantly different from the noninfested soil.

DISCUSSION

Volatile compounds released from hydrated, intact rapeseed meal had detrimental effects on each stage in the life cycle of *A. euteiches* f. sp. *pisi*. Volatiles from intact meal completely suppressed mycelial growth and encysted zoospore germination of the pathogen. In contrast, volatiles from autoclaved meal had no influence on mycelial growth and inhibited encysted zoospore germination by approximately 34%.

The predominant components of the gas phases collected from intact and autoclaved meal were nitriles and isothiocyanates. Formation of these products results from glucosinolate hydrolysis. Autoclaving the meal denatured myrosinase, but had very little effect on glucosinolate concentrations, compared with non-autoclaved tissue. The overall impact of inhibiting enzymatic catalysis is reflected both qualitatively and quantitatively in the volatile hydrolysis products released from the respective tissues. The relative concentration of volatile compounds from autoclaved meal was only 36% of that ob-

served for intact meal, presumably as a direct result of myrosinase denaturation and the subsequent reduction in glucosinolate hydrolysis rates.

Nitriles dominated the volatile profile in the autoclaved meal in contrast to isothiocyanate predominance in volatile samples evolved from intact tissues. Thermal degradation of glucosinolates during autoclaving, as opposed to enzymatic hydrolysis, enhances nitrile formation (19,30). Thus, nitriles are indeed expected as products in autoclaved meal, but not in intact meal. However, the percentage of glucosinolates non-enzymatically hydrolyzed is relatively small compared with the total glucosinolate substrate pool, because glucosinolate concentrations in both intact and autoclaved meal are similar.

These data suggest that isothiocyanates evolved from intact meal are effective inhibitors of *A. euteiches* f. sp. *pisi*. Nitriles present in gases emanating from autoclaved meal do not cause similar inhibition either because they are present at lower concentrations or because they are less toxic. Differential toxicity of specific isothiocyanate and nitrile compounds toward *A. euteiches* f. sp. *pisi* was not determined. Greenhalgh and Mitchell (13) reported that allyl nitrile showed no toxicity toward *Peronospora sporangia* even at concentrations $>1,000$ ppm. In contrast, allyl isothiocyanate was fungitoxic at concentrations as low as 10 ppm. Similarly, Lewis and Papavizas (16) demonstrated that commercially produced isothiocyanates at concentrations as low as 0.2 to 3.0 ppm completely prevented germination of encysted zoospores of *A. euteiches* f. sp. *pisi*.

Volatile compounds released from intact rapeseed meal were extremely effective in the suppression of encysted zoospore germination. However, this apparent fungicidal activity was less effective in disease suppression. The 24-h incubation of zoospore-infested peas in the atmosphere of volatile compounds from either intact or autoclaved meal decreased disease severity by 50 and 25%, respectively, compared

with controls. The effect of volatiles toward encysted zoospores may have been partly fungistatic, allowing some encysted zoospores to subsequently germinate and infect pea seedlings. It is also possible that motility of zoospores may have been reduced.

The most important propagule in the ecology of *Aphanomyces* may be the thick-walled oospore that serves as a survival structure of the pathogen. A positive correlation has been observed between oospore numbers in field soil and root rot disease severity (4,20,26). These results demonstrated toxic effects of volatile glucosinolate degradation products on oospores of *A. euteiches* f. sp. *pisi*. Exposing soil to volatile compounds from rapeseed meal significantly reduced the number of recoverable oospores and inoculum potential of those oospores. Although we speculate that oospores probably lysed in the presence of the volatile compounds, lysis was not specifically demonstrated. Techniques to quantify numbers of viable oospores in soil by use of selective media and dilution plating have not been developed. Unfortunately, enumeration of oospores with fluorescence microscopy did not provide information about the physiological condition of the oospores that were counted, although it is likely that even partially damaged oospores have lower inoculum potential.

The decision to use soil fumigation with rapeseed meal or tissues must consider, in addition to direct impacts on the pathogen, potential phytotoxicity of the tissue volatiles. Weights of plants growing from seeds incubated in the presence of volatiles from rapeseed meal were decreased, compared with controls. This is in agreement with other authors who have also observed phytotoxicity in the presence of various *Brassica* spp. (22). Volatile glucosinolate hydrolysis products have been implicated in seed germination inhibition and a reduction in overall plant growth (2).

These experiments demonstrated detrimental effects of volatile compounds re-

Table 3. Effect of volatile compounds from hydrated rapeseed meal on oospore inoculum potential of *Aphanomyces euteiches* f. sp. *pisi*

Treatment ^x	Aphanomyces rating ^y	Plant weight (g) ^z	Root weight (g) ^z
Without pathogen:	...		
No meal	...	1.63 a	0.45 a
Pathogen present	...		
No meal	3.9 a	0.87 b	0.11 b
Rs	0.2 b	1.64 a	0.46 a
ARs	0.9 b	1.43 a	0.36 a

^x Soil was either infested with oospores of *A. euteiches* f. sp. *pisi* (500/g) or not, and incubated 10 days in the presence or absence of non-autoclaved rapeseed meal (Rs) or autoclaved meal (ARs). The soil was then mixed with sterile sand and peas were planted and evaluated after 18 days.

^y Seedlings were rated on a 0 to 4 rating scale for *Aphanomyces* root rot: 0 = plant healthy; 1 = root slightly discolored; 2 = root extensively discolored but not shrunken; 3 = root extensively discolored and shrunken; and 4 = root partially or completely rotted or plant dead (24). Means (N = 50) in the same column followed by the same letter are not statistically different (least significant difference [LSD]; $P \geq 0.05$). Plants in the noninfested soil had a disease rating of zero.

^z Wet weights of the whole plant and root system were obtained for each plant. Means (N = 50) in the same column followed by the same letter are not statistically different (LSD; $P \geq 0.05$).

leased from rapeseed meal on each stage in the life cycle of *A. euteiches* f. sp. *pisi*. Although the fungicidal activity of individual glucosinolate degradation products was not determined, a direct relationship between levels of these compounds, particularly isothiocyanates, and toxic effects toward *A. euteiches* f. sp. *pisi* was established. Additional information concerning the biological activity of individual isothiocyanates, influence of these compounds on disease processes, and potential use of *Brassica* spp. for disease management should be the focus of future research.

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