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Effects of Chlorogenic Acid and Arbutin on Growth and Spore Germination of Decay Fungi

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Abstract. **Chlorogenic acid and arbutin reduced spore germination of *Penicillium expansum* Lk. ex Thom. and mycelial growth of *Mucor piriformis* Fischer. Arbutin decreased growth of *Botrytis cinerea* Per ex Fr. However, chlorogenic acid increased both germination and growth of *B. cinerea*.**

Many phenolic compounds in plant tissues are toxic to certain fungi and bacteria (2, 5, 6). Walker (10) showed that resistance of colored onions to smudge and neck rot was due primarily to toxic water-soluble phenolics in the dry outer scales of onions and that these compounds were absent or in low concentration in susceptible white-skinned cultivars. Byrde (2) suggested that the resistance to brown rot of certain high-tannin cultivars of apples was related to reduced activity of fungal-macerating enzymes by oxidized polyphenols.

Arbutin and chlorogenic acid are the major phenolics found in both the peel and flesh of 'd'Anjou' pear fruit (1, 7). This study was undertaken to investigate the effects of a range of concentrations of these phenolics on mycelial growth and conidial germination of storage-decay fungi of 'd'Anjou' pear fruit.

Cultures of *P. expansum, B. cinerea,* and *M. piriformis* were maintained on potato-dextrose agar. Spores and mycelia were obtained by incubating the cultures at 20°C. Stock solutions of arbutin and chlorogenic acid (Sigma) were added to Czapek Dox's Agar (Difco) at 50° to achieve final concentrations in the medium of 0, 100, 200, 300, 400, and 500 mg·liter^{-1}.

Mycelial growth and spore germination were evaluated relative to phenolic concentrations. From the margins of 7-day-old cultures of *P. expansum* and *B. cinerea* and 5-day-old cultures of *M. piriformis*, 4-mm-diameter plugs were cut with a cork borer. Plugs were placed upside down onto the phenolic test media in 9-cm-diameter petri dishes and incubated in darkness at 20°C. Colony diameter was measured daily thereafter until control plates were entirely covered by the fungal colony. Each phenolic concentration treatment consisted of 4 plates as replicates.

Spores from 20-day-old cultures of *P. expansum* and *B. cinerea* and 7-day-old *M. piriformis* were harvested into water, and final spore concentration was adjusted with a hemacytometer and appropriate dilutions to 10^3 conidia/ml. This suspension was sprayed onto agar media that contained the different concentrations of phenolics. Plates were incubated at 20°C. Microscopic examination at 100× was used to assess germination of spores. A spore was considered germinated if the germ tube was longer than the width of the spore. Counts were made at 2-hr intervals up to 18 hr after placing the spores on the media. A minimum of 100 spores were counted on each plate. Each treatment consisted of 4 replications.

B. cinerea mycelial growth increased as chlorogenic acid concentration increased (Table 1) but decreased with increasing arbutin levels (Table 2). Both arbutin and chlorogenic acid significantly decreased growth of *M. piriformis* (Tables 1 and 2). Mycelial growth of *P. expansum* on media containing chlorogenic acid or arbutin was not affected by either phenol (Tables 1 and 2). Mycelial growth of *P. expansum, B. cinerea*, and *M. piriformis* reached the edge of the 9-cm control plates in 12, 10, and 5 days, respectively.

Chlorogenic acid increased germination of conidia of *B. cinerea* (Table 1) but arbutin did not affect germination (Table 2). Neither arbutin nor chlorogenic acid affected germination of *M. piriformis* spores (Tables 1 and 2). However, both arbutin and chlorogenic acid decreased germination of *P. expansum* conidia (Tables 1 and 2). Growth and germination data in Tables 1 and 2 were from selected observation times that demonstrated representative effects.

It was apparent from these studies that the effects of chlorogenic acid and arbutin on fungal growth and germination were complex. For example, mycelial growth of *B. cinerea* was increased with chlorogenic acid but decreased with arbutin. Also, while chlorogenic acid increased growth of *B. cinerea*, it decreased growth of *M. piriformis*. Because of these specific effects, caution must be used when interpreting data involving total phenols rather than specific compounds. Additional research is necessary to elucidate the net effect of the naturally occurring complexes of phenolic compounds in plant resistance to fungal pathogens.

Cruickshank and Perrin (3) indicated that fungal toxicity of phenolics that are present in low concentrations may be negligible; they may, in fact, have a stimulatory effect on fungi if the concentration is low enough. Taliyeva (9) found that anthocyanin from onion

Table 1. Effects of chlorogenic acid on spore germination and growth of *B. cinerea, M. piriformis,* and *P. expansum*.

Chlorogenic acid (mg·liter^{-1})	Growth (mm)[z]			Germination (%)[y]		
	B. cinerea	*M. piriformis*	*P. expansum*	*B. cinerea*	*M. piriformis*	*P. expansum*
0	54.3	41.0	63.2	37.5	47.7	46.5
100	57.4	40.2	61.5	37.0	50.4	47.0
200	56.4	39.2	62.5	44.0	44.5	44.0
300	59.5	38.7	62.7	47.9	50.9	45.0
400	60.2	37.1	60.9	50.9	50.1	37.5
500	63.7	37.5	60.9	64.8	52.2	28.5
Significant effects						
Linear regression	**	**	NS	**	NS	*
Intercept	54.50	40.93	---	34.03	---	49.81
Slope	0.017	−0.008	---	0.052	---	−0.034

[z]Each value represents colony diameter on 4 replicate plates at 20°C after 7, 2, and 7 days for *B. cinerea, M. piriformis*, and *P. expansum*, respectively.
[y]Each value represents germination of 100 spores per plate on 4 replicate plates at 20°C after 6, 8, and 16 hr for *B. cinerea, M. piriformis*, and *P. expansum*, respectively.
**,*,NSLinear regression of growth or germination on chlorogenic acid concentration significant at 1%, 5%, and nonsignificant levels, respectively.

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Table 2. Effects of arbutin on spore germination and growth of *B. cinerea*, *M. piriformis*, and *P. expansum*.

Arbutin (mg·liter^{-1})	Growth (mm)[z]			Germination (%)[y]		
	B. cinerea	*M. piriformis*	*P. expansum*	*B. cinerea*	*M. piriformis*	*P. expansum*
0	46.5	41.0	50.6	31.0	47.7	29.0
100	45.4	40.3	50.3	27.0	30.6	22.5
200	45.8	37.7	50.8	32.0	30.3	24.3
300	43.2	38.1	51.0	26.5	32.5	22.5
400	41.7	37.4	50.7	28.0	32.4	20.0
500	41.3	35.5	51.0	28.5	33.2	21.0
Significant effects						
Linear regression	**	**	NS	NS	NS	*
Intercept	46.82	40.89	---	---	---	26.73
Slope	−0.011	−0.010	---	---	---	−0.014

[z]Each value represents colony diameter on 4 replicate plates at 20°C after 6, 2, and 6 days for *B. cinerea, M. piriformis,* and *P. expansum*, respectively.
[y]Each value represents germination of 100 spores per plate on 4 replicate plates at 20°C after 6, 8, and 16 hr for *B. cinerea, M. piriformis*, and *P. expansum*, respectively.
**,*,NSLinear regression of growth or germination on arbutin concentration significant at 1%, 5%, and nonsignificant, respectively.

bulbs stimulated germination of conidia of *B. cinerea*, while a breakdown product, anthocyanidin, was slightly toxic. In this study, we found that chlorogenic acid simulated both germination and growth of *B. cinera*. Dubernet and Ribereau-Gayon (4) concluded that *B. cinerea* possesses a polyphenol oxidase (laccase) that degrades several phenolic substances such as chlorogenic acid, anthocyanins, tannins, and vanillic acid.

From a previous study (1), the amounts of chlorogenic acid and arbutin in pear fruit were low, about 12–60 mg·liter^{-1} in flesh tissues and 240–480 mg·liter^{-1} in peel tissues. Although the phenolic amounts in flesh may be too low to have inhibitory effects on decay pathogens, the phenolics in peel are in the approximate concentration range found to be inhibitory to certain fungi in this study. Spotts (8) reported that *B. cinerea, M. piriformis*, and *P. expansum* caused decay of detached, wounded 'd'Anjou' and 'Bartlett' pear fruit but not of nonwounded fruits. Thus, the role of specific phenolics in resistance of pear fruit to certain decay fungi merits further study, particularly where mechanical injuries do not penetrate very deeply beneath the skin surface.

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Influence of Simulated Shipping on the Interior Performance of Poinsettias

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Abstract. **'Gutbier V-10 Amy' ('Amy') poinsettia lost more leaves and cyathia after simulated shipping at different temperatures (4°, 16°, or 24°C) and 30 days under interior conditions than 'Annette Hegg Dark Red' ('AHDR') plants. 'Amy' and 'AHDR' plants lost a large number of leaves when shipped for more than 4 days at 24°. 'Amy' quality was reduced when shipped at 4° due to chilling injury (white lesions on bracts). Bracts less than 2.5 cm long were most sensitive to this injury.**

Poinsettia production has been increasing in the southeastern United States (8), and plants are transported and sold throughout the eastern United States. Plants are typically sleeved, boxed, and then shipped for 2–7 days before being sold in retail outlets. In recent years, growers have encountered problems with leaf yellowing and abscission during shipping of 'Amy' poinsettia.

Previous research has shown that shipping temperatures and duration affect postproduction quality of 'Gutbier V-14 Glory' ('Glory') (5, 6), 'AHDR', and 'Annette Hegg Supreme' (5, 6, 7) poinsettias. Best quality 'Glory' poinsettia plants were obtained at storage temperatures of 13°C, while reduced epinasty and highest quality plants were observed in the other 2 cultivars at 10°. Plant quality decreased with increased shipping duration, but only 28% of leaves abscised on 'Glory' after 6 days at 18°. Leaf yellowing and abscission have not been reported for other poinsettia cultivars following shipping. Sleeving of poinsettias prior to boxing caused leaf epinasty in some cultivars such as 'AHDR', but not 'Amy' (3). The study reported here was conducted to evaluate effects of simulated shipping temperature and duration on the interior performance of 'AHDR' and 'Amy' poinsettias.

Rooted cuttings of 'Amy' and 'AHDR' poinsettias were obtained from a commercial source on 8 Sept., planted one per 15-cm container in Metro-Mix 500 (W.R. Grace, Cambridge, Mass.), and placed in a poly-

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