Characterization of muskmelon fruit peroxidases at different developmental stages

C.L. BILES, B.D. BRUTON, J.X. ZHANG and V. RUSSO

Biology Department, East Central University, Ada, Oklahoma 74820, USA
United States Department of Agriculture, Agriculture Research Service, Lane, Oklahoma 74555, USA

Abstract

An increase in exocarp peroxidase activity was observed in fruit at 5 to 30 days post pollination (DPP), and decreased at 40 and 50 DPP. Total peroxidase activity of the mesocarp was significantly lower than the exocarp in all developmental stages. Mesocarp peroxidase activity decreased consecutively from outer, to middle and, to inner tissue at every developmental stage. Total activity in the mesocarp peaked at 20 DPP. Native-PAGE of exocarp tissue showed at least two cathodic (basic) peroxidases and two anionic (acidic) peroxidases. The number of isozymes was greatest and bands most intense at 30 DPP. IEF-PAGE of the 5 to 50 DPP fruit exocarp showed at least 8 peroxidase isozymes (pI 4.6 to 9.6). Anion exchange chromatography showed only one peak of anionic peroxidase activity that was not evident until 15 DPP. This peak was greatest at 30 DPP and declined at 40 and 50 DPP. Cationic peroxidase isozymes appeared to be the predominant and most intense isoforms throughout fruit development. The changes in peroxidase activity corresponded to fruit formation and may be associated with susceptiblity to fruit rot.

Additional key words: *Cucumis melo*, chromatography, electrophoresis, host-plant resistance.

Introduction

Softening in muskmelon fruit (*Cucumis melo* L. var. *cantalupensis* Naud.) begins 28 days post-pollination (DPP) and continues through postharvest (Lester and Dunlap 1985). Fruit are ripe approximately 40 DPP. Lester and Dunlap (1985) found a 400 % decrease in tissue firmness between 30 and 50 DPP. During this same period, latent and non-latent infecting fungi can render fruit unmarketable (Bruton 1995, Webster and Craig 1976).

Little is known about the mechanism of latency in postharvest pathogen-fruit interactions. Peroxidases (EC 1.11.1.7) are generally known to be a stress induced enzymes that have been implicated in plant defense mechanisms. This family of enzymes has been associated with disease resistance, wound healing, lignification, phenol polymerization, suberization, protection against H_2O_2 and other oxidants, the oxidative burst of the hypersensitive response (Martinez *et al.* 1998), drought tolerance, chlorophyll degradation, senescence, and cuticle development. Peroxidases also have been reported to have antifungal activity (Peng and Kuć 1992). Activity of peroxidase increases as cucumber fruit matures (Repka and Fischerová 1996, Miller *et al.* 1989), and as cucumbers are mechanically stressed (Miller and Kelley 1989). Peroxidase has been suggested as a marker enzyme to recognize resistant muskmelon cultivars (Lebeda *et al.* 1999, Reuveni *et al.* 1992). Smith and Hammerschmidt (1988) found that a family of anionic peroxidases systemically enhanced in watermelon, cucumber, and muskmelon when leaves were inoculated with *Colletotrichum lagenarium*. The induced resistance response has been correlated with increased peroxidase levels in cucurbits by many workers (Biles and Martyn 1993, Dalisay and Kuć 1995, Rasmussen *et al.* 1995, Smith and Hammerschmidt 1988).

Received 23 November 1999, *accepted* 12 March 2000.
Abbreviations: BSA - bovine serum albumin; DPP - days post-pollination; HPLC - high pressure liquid chromatography; IEF-PAGE - isoelectric focusing-polyacrylamide gel electrophoresis; SDS-PAGE - sodium dodecyl sulfate-polyacrylamide gel electrophoresis.
Acknowledgements: The authors thank Diann Baze, Anthony Dillard, Scott Linthicum, and Tracy Pardue for excellent technical assistance. This research was supported by NIH-Bridges to the Baccalaureate grant (GM-96-001), USDA Cooperative Grant (Agreement no. 58-6222-6-018), and East Central University.
Fax: (+1580) 310 5606, e-mail: cbiles@mailclerk.ecol.edu

Although, peroxidases have been associated with many plant functions, the precise role for peroxidase in stress tolerance, plant structure development, and repair is not fully understood. Information on the role of peroxidase in fruit development will aid in determining its possible function in disease resistance and ripening. The objective of this research was to characterize muskmelon peroxidases during fruit development.

Materials and methods

Muskmelon fruit: Muskmelon plants (*Cucumis melo* L. var. *cantalupensis* Naud cv. Magnum-45) were grown in experimental field plots in Lane, Oklahoma, USA, in the summer of 1998 according to standard practices. Female flowers were tagged as soon as they were receptive for pollination and fruit were harvested at developmental stages at 5, 10, 15, 20, 30, and 40 (horticultural maturity) DPP. Additional fruit were harvested at 40 DPP and stored 10 d at 4 °C, hereafter referred to as 50-day fruit. Tissue plugs (18-mm in diameter) were removed from 4 melons (15 plugs each) at each developmental stage. Each plug was dissected into the exocarp (~3 mm width), outer mesocarp (~10 mm width), middle mesocarp (~10 mm width), and inner mesocarp (~10 mm width) and stored at -20 °C until the proteins were extracted.

Preparation of fruit tissue extracts: Fruit proteins were extracted by homogenizing 10 g of tissue in 10 cm^3 of 0.1 M Na-acetate buffer (pH 5) containing 1 M NaCl and 1 mM of EDTA according to Zhang *et al.* (1997). The homogenates were centrifuged at 32 000 *g* for 15 min, and the supernatant collected and stored at -20 °C until used in experiments. Samples applied to Native- and IEF-PAGE or HPLC (*BioRad, Biologic system*, Richmond, CA, USA) were dialyzed against 25 mM TRIS-HCl buffer, pH 7.8 for 24 h. Protein content was measured with the *BioRad* protein assay reagent according to instructions of the manufacturer. Bovine serum albumin was used as a standard.

Peroxidase assay: Spectrophotometric readings (absorbance at 470 nm) were recorded 30 s to 2 min after 0.025 to 0.10 cm^3 of sample were added to 1 cm^3 of 10 mM guaiacol and 10 mM H_2O_2 in 50 mM Na-acetate buffer, pH 5.0. Peroxidase activity was linear within the time and sample aliquot ranges. Experiments consisted of 3 replications per maturity group. One unit of muskmelon peroxidase activity was defined as 1.4 absorbance (at 470 nm) units which was determined with 1 unit of horseradish peroxidase (*Sigma Chemical Co.*, St. Louis, USA) in 10 mM guaiacol and 10 mM H_2O_2 in 50 mM Na-acetate buffer, pH 5 for 1 min.

Tissue blotting: Tissue blotting of muskmelon fruit was conducted according to the procedures previously described (Biles *et al.* 1993, Spruce *et al.* 1987). Nitrocellulose filters (*Bio-Rad*) were floated on 50 mM Na acetate buffer, pH 4.5. Filters were blotted on a paper towel until semidry. Muskmelons were harvested at 5, 10, 15, 20, 30, 40, and 50 DPP, respectively. Transverse sections (5 mm) of the fruit exocarp and mesocarp were placed on the filters for 5 min. Fruit pieces were removed, and filters were stained with 10 mM guaiacol and 10 mM H_2O_2 in 50 mM Na acetate buffer, pH 4.5.

Gel electrophoresis: Dialyzed protein extracts (4 µg of total protein per lane) were applied to a 4 % loading and 12 % separation PAGE (0.75 mm thick) without sodium dodecyl sulfate, according to the instructions provided by *BioRad*. Gels were electrophoresed with 200 V (constant voltage) for 2 h at 4 °C and immediately stained for peroxidase activity with 10 mM guaiacol and 10 mM H_2O_2. A bromophenol tracking dye was added to the samples before they were loaded onto the gel.

IEF-PAGE was also used to separate the fruit peroxidase isozymes. Proteins were extracted and dialyzed as described above, and samples were placed on *Whatman No. 1* wicks (5 × 5 mm). The wicks were saturated with fruit extract (2 µg protein per lane) and placed in the middle of a 5 % IEF-PAGE gel containing a pH 3 - 10 ampholyte (*Pharmalyte* 3-10 for IEF, *Pharmacia Biotech AB*, Uppsala, Sweden) gradient. Separations in the gel were conducted for 20 min at 100 V. The wicks were then removed, and the gel was electrophoresed for an additional h at 400 V. All procedures were followed as described by the manufacturer, except for modified electrophoretic time, for operation of the model *111 Mini-IEF Cell* (*BioRad*). Immediately after removing the gel from the cell chamber, the gel was stained for peroxidase activity bands as described above. Isoelectric points were determined by using a set of broad range IEF standards in the range of pI 4.45 - 9.6 (*BioRad*).

HPLC analysis: Native, soluble proteins (approximately 200 µg) extracted from exocarp tissue of muskmelon fruit at each developmental stage were applied to a *Bio-Scale* Q2 anion exchange column, 7 mm × 52 mm (*Bio-Rad*, Hercules, USA) attached to a *Biologic* chromatography system (*Bio-Rad*) in order to partially purify the peroxidase isozymes and determine the relative charge of the enzymes. The column was equilibrated with 25 mM Tris-HCl, pH 7.8. A gradient of 0 to 1 M NaCl in the same buffer was used to elute the anionic peroxidases from the Q2 column. Fractions were collected each minute and the gradient was run for 30 min. Each fraction was assayed for peroxidase activity as previously described.

Results

Peroxidase activity in tissue: Peroxidase activity was detected in all tissues types throughout fruit development (Fig. 1*A*, *B*). Peroxidase activity in exocarp tissue was 2 to 57-fold higher than any portion of mesocarp tissue, with the highest peroxidase activity found in fruit at 30 DPP (Fig. 1*A*). Whereas, peroxidase activity increased in fruit exocarp from 5 to 30 DPP, peroxidase activity of mesocarp tissue was greatest in fruit at 20 DPP (Fig. 1*B*). Outer mesocarp tissue had the highest peroxidase activity in all developmental stages, followed by middle, and inner mesocarp, respectively.

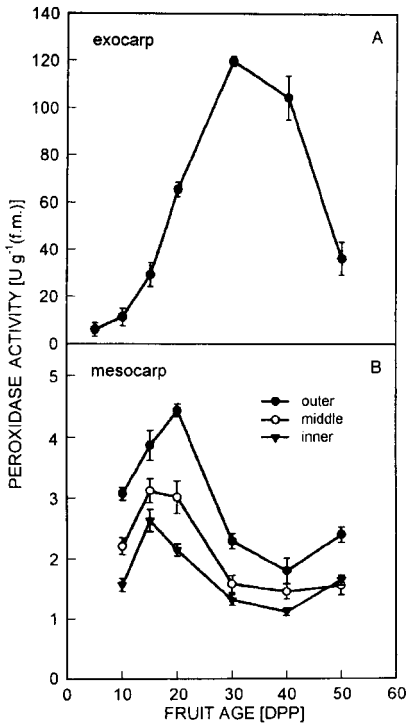

Fig. 1. Total peroxidase activity in muskmelon exocarp (*A*) and outer, middle, and inner mesocarp (*B*) in fruit 5 through 50 DPP. Five-day mesocarp was bulked due to the small quantity and was not included in the graph (*B*). Mean peroxidase activity of the 5-day mesocarp was 2.17 ± 0.051. *Vertical bars* represent SE.

Nitrocellulose blots of muskmelon tissue stained for peroxidase activity showed that peroxidase was present in all tissues throughout development (Fig. 2). Intensity of peroxidase tissue blots was greater in exocarp tissue in 15 through 50 DPP fruit when compared with that of mesocarp tissue.

Peroxidase isozymes of fruit exocarp: Native-PAGE showed a cathodic band in fruit exocarp at 15 DPP, which increased as the fruit developed until 40 DPP (Fig. 3). Another cathodic band at R_f 0.09, was observed in fruit exocarp at 30, 40, and 50 DPP. Two anionic peroxidases were observed at R_f 0.48 and 0.54. These peroxidase isozymes were most intense in fruit exocarp at 20, 30, and 40 DPP. They were not evident in fruit at 50 DPP. Extracts were loaded according to fruit fresh mass. Similar results were observed when extracts were loaded based on protein content of samples (data not shown).

IEF-PAGE showed at least nine peroxidase isozymes with pI's of 4.6, 5.1, 5.2, 6.3, 7.0, 7.7, 7.8, 8.5, and 9.6 in fruit exocarp during fruit development (Fig. 4). These isozymes increased as fruit developed and peaked in fruit

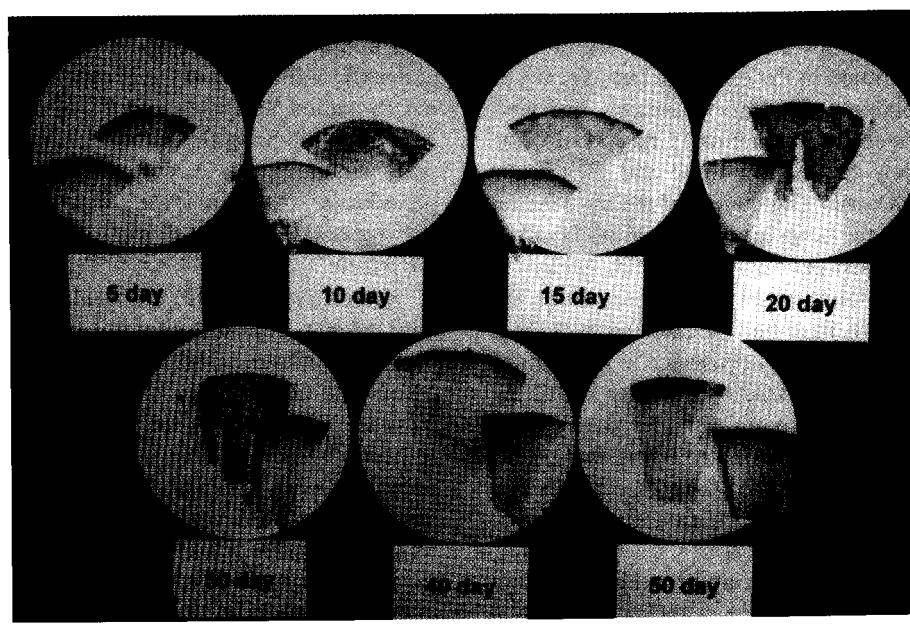

Fig. 2. Nitrocellulose tissue blotting of muskmelon tissue at 5 - 50 DPP. The lower side of each nitrocellulose disk shows the tissue blotted. After blotting, the disk was submerged in 10 mM guaiacol and 10 mM H_2O_2 to identify location of peroxidase activity in the fruit.

exocarp at 30 DPP, and decreased slightly in 40 and 50 day fruit exocarp. The peroxidase isozyme with a pI of 8.5 was present throughout fruit development and was the most prominent isozyme in regard to band intensity. The isozymes with pIs of 4.6, 5.1, and 5.2 were not as evident in fruit tissue at 50 DPP. Gel samples were loaded based on protein content, but similar results were obtained when samples were loaded based on tissue fresh mass.

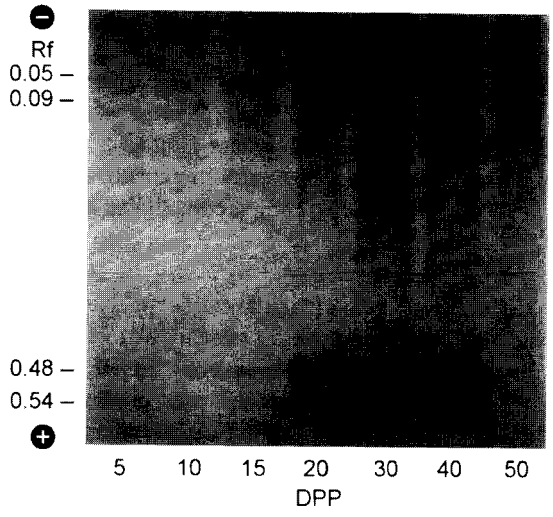

Fig. 3. Native-PAGE (10 - 15 % gradient) of muskmelon exocarp peroxidases from 5 - 50 DPP. Gels were stained in 10 mM guaiacol and 10 mM H_2O_2 to visualize peroxidase isozymes.

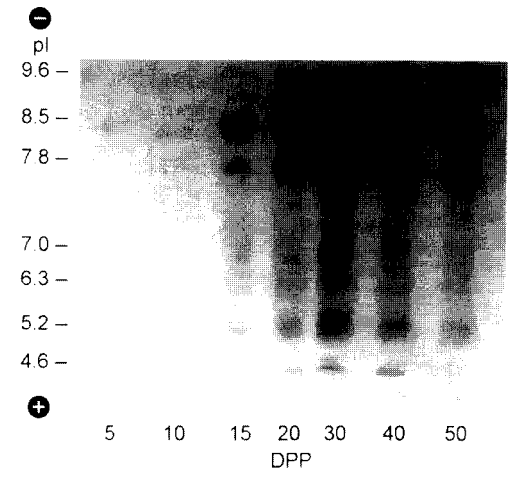

Fig. 4. IEF-PAGE (5 % gel) of muskmelon exocarp peroxidases from 5 - 50 DPP. Gels were stained in 10 mM guaiacol and 10 mM H_2O_2 to visualize peroxidase isozymes.

HPLC analysis: Anion-exchange chromatography indicated very low exocarp peroxidase activity until 15 DPP. The largest peroxidase peak was first eluted from the column before the salt gradient was applied, and this peak was observed in fruit from 15-50 DPP (Fig. 5). A small anionic peroxidase activity peak was also observed in fruit at 15 DPP (Fig. 5). This anionic peak increased approximately 4-fold in fruit exocarp at 30 DPP and decreased in fruit exocarp at 40 and 50 DPP.

Discussion

Muskmelon peroxidases were present in both exocarp and mesocarp tissues thoughout fruit development, but were in much higher concentration in the exocarp. The exocarp of muskmelon is an elaborate system of lenticels and their complementary cork cells (Webster and Craig 1976). The cork cells are closely packed, air-filled and their walls contain waxes and suberin. The cuticle of the muskmelon exocarp is located on the outer and anticlinal walls of the epidermis, and consists primarily of cutin, which is composed of highly polymerized fatty acids. Both the cuticle and the cork cells have been implicated in defense against pathogen penetration and resistance to mechanical damage. However, as emphasized by Webster and Craig (1976), the cuticle is extensively ruptured during fruit enlargement allowing ingress of insects and pathogens. Once the net is fully formed (25 to 30 DPP), resistance to the pathogen infection appears to increase (Bruton 1995, Davis *et al.* 1965).

This study concentrated on exocarp peroxidases because of their implicated role in suberization and cuticle formation (Biles *et al.* 1993, Ebermann and Pichorner 1989, Mohan and Kolattukudy 1990). Peroxidase activity in muskmelon exocarp was highest at 30 DPP, and then decreased as the fruit continued to develop. The increase in peroxidase activity (15-30 DPP) in the exocarp corresponds to active net formation (Webster and Craig 1976, Bruton 1995). Nitrocellulose blots also indicated that peroxidase activity was highest in the exocarp. These results suggest that peroxidase plays a role in the suberization and polymerization of cell wall-associated phenolic polymers of the cuticle.

Bruton *et al.* (1998) reported that wound and latent muskmelon polygalacturonases macerated exocarp less than mesocarp tissue. Our study indicated that exocarp peroxidase activity was *ca.* 16 and 52-fold higher than mesocarp peroxidases of fruit at 20 and 30 DPP, respectively. In addition, Bruton *et al.* (1998) noted that outer mesocarp was macerated less than inner mesocarp when exposed to fungal polygalacturonases. In the current study, peroxidase activity was lower in the inner mesocarp tissue when compared to the middle and outer mesocarp at every developmental stage tested, except in fruit harvested at 50 DPP in which inner and middle mesocarp were not significantly different. Recently, Zhang *et al.* (1999)

reported that muskmelon fruit at 10 DPP or less was more susceptible to the Black rot pathogen, *Didymella bryoniae*, compared to more mature fruit. The current study indicated that the increase of peroxidase occurred in fruit at 15 and 20 DPP. This body of information suggests that the muskmelon fruit tissues that have a high level of peroxidase activity appear to be less susceptible to maceration by fungal pathogens.

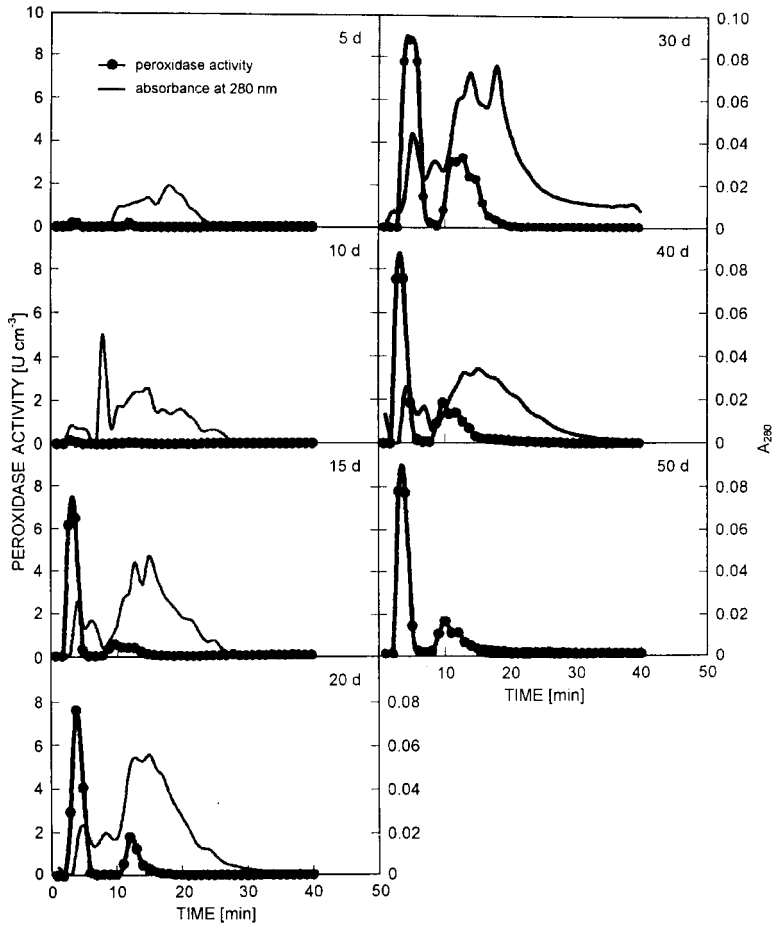

Fig. 5. Peroxidase profiles of the anion-exchange chromatography of the proteins extracted from muskmelon exocarp fruit tissue harvested 5 - 50 DPP.

Latent fungal pathogens are inactive in muskmelon fruit until 40 - 50 DPP (Bruton 1995, Bruton *et al.* 1998, Zhang *et al.* 1997). The increase in peroxidase activity during fruit development appears to correspond with the latent period of postharvest pathogens, such as *P. cucurbitae* (Bruton *et al.* 1998, Zhang *et al.* 1997). The decrease in fruit peroxidases at 40 and 50 DPP appears to correspond with the end of latency and onset of postharvest decay. Lacan and Baccou (1998) also observed a decrease in muskmelon fruit peroxidases after harvest.

Anionic peroxidase have been induced by wounding (Ride 1975), pathogen attack in cucurbits (Smith and Hammerschmidt 1988, Biles and Martyn 1993), ripening in watermelons and peppers (Biles and Martyn 1993, Biles *et al.* 1997) and normal developmental processes of cucumber (Repka and Fischerová 1996). Muskmelon peroxidases were present in both exocarp and mesocarp in all developmental stages and highly concentrated in the exocarp from 15 to 30 DPP. Peroxidase appears consistently in the exocarp of several fruits, implying a role in suberization and lignification of these tissues (Biles *et al.* 1993, Repka and Fischerová 1996, Calderón *et al.* 1993). The highest rate of peroxidase accumulation was found in the epidermis of cucumber (Repka and Fischerová 1996). They observed a decreasing gradient of peroxidase activity from the epidermis to the inner placenta region of the fruit, as was found in muskmelon fruit in our study. In addition activity of stress-related anionic peroxidases were found to increase as the fruit matured. Miller and Kelley (1989) found that the fastest moving peroxidase isozyme (anodic) became more active with storage of the cucumbers after mechanical stress. They postulated that the biological role of peroxidase activity following mechanical stress may be the involvement in wound-healing processes. If net development can be considered analogous to wounding because of breaking of the epidermis, then it appears the

anionic forms of peroxidase may play a major role in the wound-stress response during suberization and lignification of the exocarp. Although many reports mentioned in this paper emphasize the role of anionic peroxidase in response to physical and chemical stress, cationic peroxidase appear to carry out many of the same processes.

Prestamo and Manzano (1993) observed a range of isozymes and molecular masses (36 - 120 kD) for peroxidases in several fruits and vegetables. Our preliminary results indicate that the cationic peroxidases of muskmelon have a molecular mass of 48 and 55 kD and anionic peroxidases 32 and 42 kD (Biles *et al.* 1998). Muskmelon anionic peroxidases appear to be similar in size to the anionic peroxidases observed in cucumber, muskmelon, and watermelon leaf tissue (ca. 30 kD) (Smith and Hammerschmidt 1988) and the well characterized tobacco leaf anionic peroxidase (36 kD) (Lagrimini *et al.* 1987, 1990).

Although cationic peroxidase are the predominant isoform in muskmelon fruit exocarp, the anionic isoforms appear to coincide with net development and dormancy in latent infections. Additional research will be required to elucidate the effects of specific peroxidase isozymes on net development and their possible role in latent infections.

References

Biles, C.L., Bruton, B.D., Zhang, J., Russo, V.: Purification and characterization of cantaloupe exocarp peroxidases. - Proc. Oklahoma Acad. Sci. **78**: 138, 1998.

Biles, C.L., Martyn, R.D.: Peroxidase, polyphenoloxidase, and shikimate dehydrogenase isozymes in relation to tissue type, maturity and pathogen induction of watermelon seedlings. - Plant Physiol. Biochem. **31**: 499-506, 1993.

Biles, C.L., Wall M.M., Waugh M., Palmer H.: Relationship of *Phytophthora* fruit rot to fruit maturation and cuticle thickness of New Mexican-type peppers. - Phytopathology **83**: 607-611, 1993.

Biles, C.L., Kuehn, G.D., Wall, M.M., Bruton, B.D., Wann, E.V.: Characterization of chile pepper fruit peroxidases during ripening. - Plant Physiol. Biochem. **35**: 273-280, 1997.

Bruton, B.D.: Etiology, epidemilogy, and control of muskmelon fruit rots. - In: Lester, G.E., Dunlop, J.R. (ed.): Proceeding of Cucurbitaceae 94. Pp. 48-54. Gateway Printing, Edinburg 1995.

Bruton, B.D., Conway, W.S., Gross, K.C., Zhang, J.X., Biles, C.L., Sams, C.E.: Polygalacturonase of a latent and wound postharvest fungal pathogen of muskmelon fruit. - Postharvest Biol. Technol. **13**: 205-214, 1998.

Calderón, A.A., Zapata, J.M., Muñoz, R., Barceló, A.R.: Localization of peroxidase in grapes using nitrocellulose blotting of freezing/thawing fruits. - HortScience **28**: 38-40, 1993.

Dalisay, R.F., Kuć, J.A.: Persistence of induced resistance and enhanced peroxidase and chitinase activities in cucumber plants. - Physiol. mol. Plant Pathol. **47**:315-327, 1995.

Davis, G.N., Whitaker, T.W., Bohn, G.W., Kasmire, R.F.: Muskmelon production in California. - Calif. Agr. Exp. Sta. Bull. **536**, 1965.

Ebermann, R., Pichorner H.: Detection of peroxidase catalyzed phenol polymerization induced by enzymatically reduced paraquat. - Phytochemistry **28**: 711-714, 1989.

Lacan, D., Baccou, J.: High levels of antioxidant enzymes correlate with delayed senescence in nonnetted muskmelon fruits. - Planta **204**: 377-382, 1998.

Lagrimini, L.M., Bradford, S., Rothstein, S.: Peroxidase-induced wilting in transgenic tobacco plants. - Plant Cell **2**: 7-18, 1990.

Lagrimini, L.M., Rothstein, S.: Tissue specificity of tobacco peroxidase isozymes and their induction by wounding and tobacco mosaic virus infection. - Plant Physiol. **84**: 438-442, 1987.

Lebeda, A., Kistkova, E., Dolal, K.: Peroxidase isozyme polymorphism in *Cucurbita pepo* cultivars with various morphotypes and diferent level of field resistance to powdery mildew. - Scientia Hort. **81**: 103-112, 1999.

Lester, G., Dunlap, J.R.: Physiological changes during development and ripening of 'Perlita' muskmelon fruits. - Scientia Hort. **26**: 323-331, 1985.

Martinez, C., Montillet, J.L., Bresson, E., Agnel, J.P., Aai, G.H., Daniel, J.F., Geiger, J.P., Nicole, M.: Apoplastic peroxidase generates superoxide anions in cells of cotton cotyledons undergoing the hypersensitive reaction to *Xanthomonas campestris* pv. *malvacearum* race 18. - Mol. Plant-Microbe Interact. **11**: 1038-1047, 1998.

Miller, A.R., Dalmasso, J.P., Kretchman, D.W.: Developmental variation of cell wall degrading enzymes from cucumber (*Cucumis sativus*) fruit tissues. - Can. J. Bot. **67**: 817-821, 1989.

Miller, A.R., Kelley, T.J.: Mechanical stress stimulates peroxidase activity n cucumber fruit. - HortScience **24**: 650-652, 1989.

Mohan, R., Kolattukudy, P.E.: Differential activation of expression of a suberization-associated anionic peroxidase gene in near-isogenic resistant and susceptible tomato lines by elicitors of *Verticillium albo-atrum.* - Plant Physiol. **92**: 276-280, 1990.

Peng, M., Kuć, J.: Peroxidase-generated hydrogen peroxide as a source of antifungal activity *in vitro* and on tobacco leaf disks. - Phytopathology **82**: 696-699, 1992.

Prestamo, G., Manzano, P.: Peroxidases of selected fruits and vegetables and the possible use of ascorbic acid as an antioxidant. - HortScience **28**: 48-50, 1993.

Rasmussen, J.B., Smith, J.A., Williams, S., Burkhart, W., Ward, E., Somerville, S.C., Ryals, J., Hammerschmidt, R.: cDNA cloning and systemic expression of acidic peroxidases associated with systemic acquired resistance to disease in cucumber. - Physiol. mol. Plant Pathol. **46**: 389-400, 1995.

Reuveni, R., Shimoni, M., Karchi, Z., Kuć, J.: Peroxidase activity as a biochemical marker for resistance of muskmelon (*Cucumis melo*) to *Pseudoperonospora cubensis*. - Phytopathology **82**: 749-753, 1992.

Repka, V., Fischerová, I.: Distribution of the stress-related anionic peroxidase in different cucumber organs. - Biol. Plant. **38**: 571-583, 1996.

Ride, J.P.: Lignification in wounded wheat leaves in response to

fungi and its possible role in resistance. - Physiol. Plant Pathol. **5**: 125-134, 1975.

Smith, J.A., Hammerschmidt, R.: Comparative study of acidic peroxidases associated with induced resistance in cucumber, muskmelon and watermelon. - Physiol. Plant Pathol. **33**: 255-261, 1988.

Spruce, J., Mayer, A.M., Osborne, D.J.: A simple histo-chemical method for locating enzyme in plant tissue using nitrocellulose blotting. - Phytochemistry **26**: 2901-2903, 1987.

Webster, B.D., Craig, M.E.: Net morphogenesis and characteristics of the surface of muskmelon fruit. - J. amer. Soc. hort. Sci. **101**: 412-415, 1976.

Zhang, J., Bruton, B.D., Biles, C.L.: Polygalacturonase isozymes produced by *Phomopsis cucurbitae* in relation to postharvest decay of cantaloupe fruit. - Phytopathology **87**: 1020-1025, 1997.

Zhang, J., Bruton, B.D., Miller, M.E., Isakeit, T.: Relationship of developmetnal stage of cantaloupe fruit to black rot susceptibility and enzyme production by *Didymella bryoniae*. - Plant Disease **83**: 1025-1032, 1999.