

SHORT COMMUNICATION  
WOUND TYPE IN APPLE FRUITS AFFECTS WOUND RESISTANCE TO  
DECAY-CAUSING FUNGI

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SUMMARY

'Golden Delicious', 'Granny Smith' and 'Red Delicious' apple fruits were wounded at their equators to give one puncture wound (3 mm diameter x 3 mm deep) and one shallow-slice wound (4.0 mm diameter x 0.4 mm deep) on opposite sides of a fruit. Freshly-made wounds (0 day old) or wounds that were 1 day or 3 days old prior to inoculation were inoculated with 50 or 500 conidia per wound of *Botrytis cinerea* or *Penicillium expansum*. After 7 days incubation at 22°C, 1 day or 3 day-old puncture wounds had less decay severity and incidence from either fungus than 0 day-old puncture wounds. Conversely, 0 day-old slice wounds were more resistant to fungal decay, whereas 3 day-old wounds were less resistant. This wound-type effect was found with both inoculum densities and for all cultivars. It occurred in 'Golden Delicious' fruits after incubation for 30 days at 3°C. This is the first report of wound type affecting the outcome of fungal infection in apple fruits. Understanding this effect may prove useful in devising a comprehensive theory of host resistance to fungal infection in apple fruit.

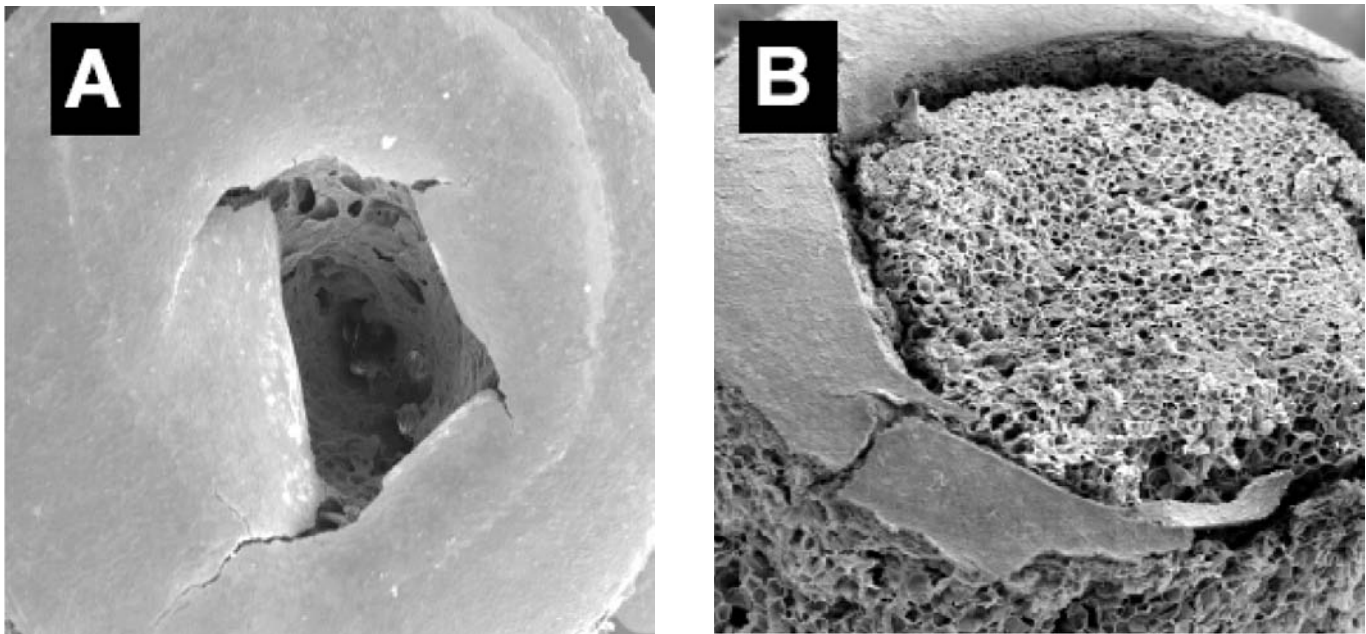
*Key words:* *Botrytis cinerea*, *Penicillium expansum*, postharvest apple diseases, apple fruit wounds, disease resistance.

Wounds in apple fruit are primary infection sites for *Botrytis cinerea* Pers.:Fries and *Penicillium expansum* Link, fungi that cause gray mold and blue mold, respectively (Rosenberger 1990; Prins *et al.*, 2000). These diseases are particular problems in the post-harvest storage of apple fruits. For the study of these diseases, wounds in apple fruits have been artificially inflicted using numerous techniques; however, puncture wounds made typically with a metal carpenter's nail or a similar puncture device have been most frequently used (Lakshminarayana *et al.*, 1987; Roberts, 1990; Janisiewicz *et al.*,

1994; Filonow, 1998).

It is generally accepted that as plant wounds age, they undergo healing, which is accompanied by the development of resistance in wounds to pathogen infection (Bostock and Stermer, 1989). Wound healing in apple fruits increased resistance in puncture wounds to infection by *B. cinerea* and *P. expansum* (Lakshminarayana *et al.*, 1987). Wounds that were inoculated within a few minutes after infliction had greater gray mold or blue mold incidence after incubation compared to wounds that were four days old prior to inoculation. In addition, wound size, host resistance, inoculum density, virulence, and environmental parameters are typically considered as factors in the expression of fungal infection of plant wounds. However, there are no reports of the effect of the configuration or type of a wound on the degree of decay in apple fruit. Casual inspections of apple fruits from supermarket shelves will show numerous wound types, such as deep punctures with outer perimeters of regular geometric form (circle, square, triangles, etc.) and shallow surface cuts with perimeters of irregular form. Therefore, the objective of this study was to determine whether the level of wound resistance to fungal decays in apple fruits is influenced by wound type prior to pathogen inoculation.

Apple fruits (*Malus x domestica* Borkh.) were purchased from local supermarkets or directly from a packing house in Michigan. 'Golden Delicious' and 'Red Delicious' fruits from Michigan were coated with wax, but were not treated with any chemicals prior to long-term controlled-atmosphere (CA) storage. 'Golden Delicious', 'Red Delicious' and 'Granny Smith' fruits from Washington were waxed, and were treated with diphenylamine (DPA) and thiabendazole (TBZ) prior to CA storage. All fruits were graded Extra Fancy Large (ca. 7.5-9.5 cm in diameter and 210-280 g). Fruits that were used in experiments were free of wounds and obvious bruises, or had minimum bruising. 'Golden Delicious', 'Red Delicious' and 'Granny Smith' fruits from Washington had a soluble solids content of 10.1-13.0%, 12.4-13.2%, or 12.0-14.2%, and a fruit firmness of 37.3-40.8 Newtons (N), 40.1-42.6 N, or 37.3-51.1 N, respectively. 'Golden Delicious' and 'Red Delicious' fruits from Michigan had a soluble solids content of 13.0-15.6%



**Fig. 1.** Scanning electron micrographs of (A) a puncture wound and (B) a slice wound from a 'Golden Delicious' apple fruit. Specimens were coated with gold-palladium and observed at 18x in a JEOL JXM 6400 SEM operating at 15 KV.

and 11.5-14.6%, and a fruit firmness of 35.5-48.0 N and 35.5-49.0 N, respectively. All fruits were stored at 3°C for no longer than two weeks before use and pre-incubated at 22°C for 16-24 h prior to wounding.

Fruits were washed in 0.5% NaOCl (v/v) for 1-2 min, rinsed in tap water and dried with paper towels. The method of Filonow (2004) was adapted to wound the fruits. For puncture wounds a single cone-shaped puncture (3 mm in diameter x 3 mm deep; conical area of 15.8 mm<sup>2</sup>) was made with the conical end of a nail in the middle of a fruit. The wounded area was immediately washed with tap water and dried with a paper towel. One slice wound was made in the middle of the same fruit, but on the opposite side by imprinting a 4.0 mm diameter circle in the fruit, without puncturing the skin. The perimeter of the circle was cut with the tip of an X-ACTO knife just below the skin (approx. 0.4 mm deep). A flexible, thin-blade, double-edge razor was used to cut under the skin, and the excised tissue was lifted off and discarded. The resulting wound was a circular-shaped cavity of about 17.6 mm<sup>2</sup> (Fig. 1). The wounded area was washed with tap water and wiped dry. Wounds were allowed to age at 22°C and 45-55% relative humidity for 3 days (d) or 1 day on a lab bench before they were inoculated. Fresh wounds (0 day old) were inoculated within 3 min of wounding.

*P. expansum* Link (PE 1) and *B. cinerea* Pers.:Fries (F-J-4) were isolated from apple. Fungal cultures were stored on silica gel and conidia produced on potato-dextrose agar (Filonow *et al.*, 1996). Fresh suspensions of 5·10<sup>3</sup> or 5·10<sup>4</sup> conidia ml<sup>-1</sup> of sterile distilled water were prepared (Filonow 2004) for each experiment and

used within 30 min of preparation.

Wounds that were 0d, 1d, or 3d-old in fruits of each cultivar were inoculated with a 10 µl gel-loading pipet tip which deposited 50 or 500 conidia per wound. Care was taken not to re-injure the 1d or 3d-old wounds with the tip. Fruits were seated at the calyx ends on plastic Petri dishes and enclosed in a 60×90×14 cm plastic box, which was lined with absorbent paper and filled with 700 ml of water. Each box held nine fruits, one each for a cv × age interaction (3 cvs × 3 ages), with the fruits randomly arranged within the box. A randomized, complete block design was used with 15 boxes (replicates) for each experiment. Decay from *B. cinerea* conidia was compared to decay from *P. expansum* conidia in separate experiments. After seven days at 22°C the wounds were measured for decay incidence (percentage of fruits in a treatment with decay) and the diameter of decay.

Using the above protocol, resistance to decay in wounds was assessed at 50 conidia per wound with *B. cinerea* in one set of experiments and with *P. expansum* in a separate set. 'Golden Delicious' and 'Red Delicious' fruits from Michigan and 'Granny Smith' fruits from Washington were used in these experiments. Resistance to each fungus was also assessed at 500 conidia per wound in separate experiments. Fruits from Washington were used in these experiments.

The effect of wound type on decay from *B. cinerea* or *P. expansum* was assessed in separate experiments inside a cold room at 3°C. Experiments were conducted only at 500 conidia per wound and only in wounds of 'Golden Delicious' fruits. The protocol was the same as previously described. Every box held three fruits, one each with

0d, 1d, and 3d-old wounds. There were 15 boxes completely randomized. Disease was measured after 30 days.

Diameters and incidences of gray mold and blue mold at 22°C in fruits inoculated with 50 conidia/wound were greatest in fresh puncture wounds than in 1d-old and 3d-old puncture wounds (Fig. 2 and 3). Conversely, both fungal decays were the lowest in slice wounds compared to greater decay levels in older wounds (Fig. 2 and 3). Wounds inoculated with 500 conidia (Fig. 4) exhibited similar decay reactions. Decay diameter and incidence caused by either pathogen in puncture wounds were greatest in fresh wounds and least in 3d-old wounds. The converse was found in slice wounds.

Decay diameter and incidence in wounds of fruits stored at 3°C for 30 days (Fig. 5) were greatest in fresh wounds and least in 3d-old puncture wounds. The opposite effect was observed with slice wounds.

Findings from the present work support those of Lakshiminarayana *et al.* (1987) who reported that fresh puncture wounds of ‘Golden Delicious’ and ‘Granny Smith’ fruits were more susceptible to gray mould or blue mould than 4d-old wounds, which showed histological evidence of wound healing. Healing periods longer than four days did not increase resistance to decay.

This is the first report of wound type affecting wound resistance to decay-causing pathogens in apple fruits. Moreover, a literature search failed to find a previous report describing this effect in any plant. The cause of this effect is unknown. The effect did not appear to be a peculiar event restricted to narrow conditions. It was observed in fruits of three apple cultivars, using two different fungal species and two different inoculum densities. The densities used in the present study were realistic for those found in packinghouses (Blanpied and Purnasiri, 1968; Rosenberger *et al.*, 1991;

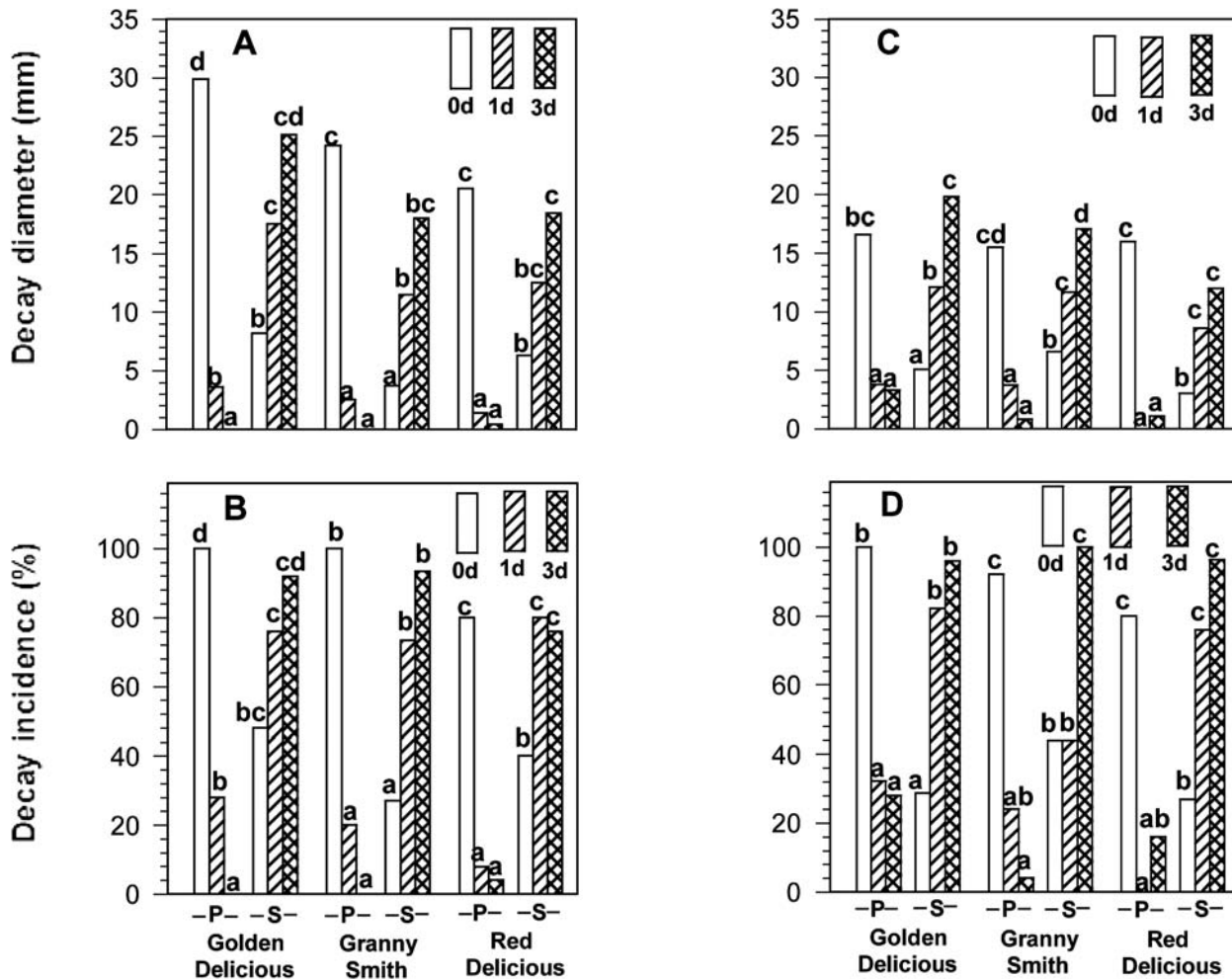
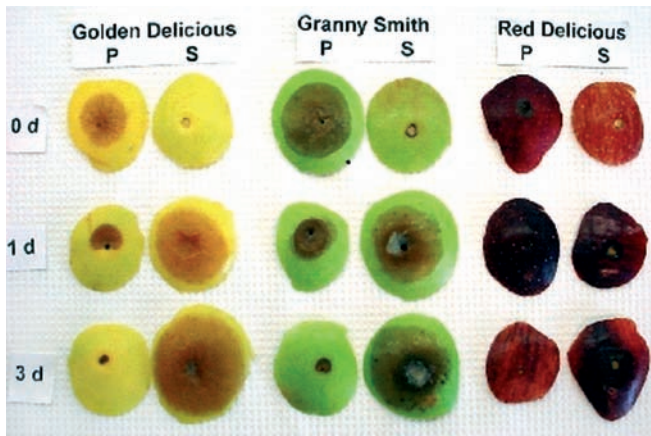


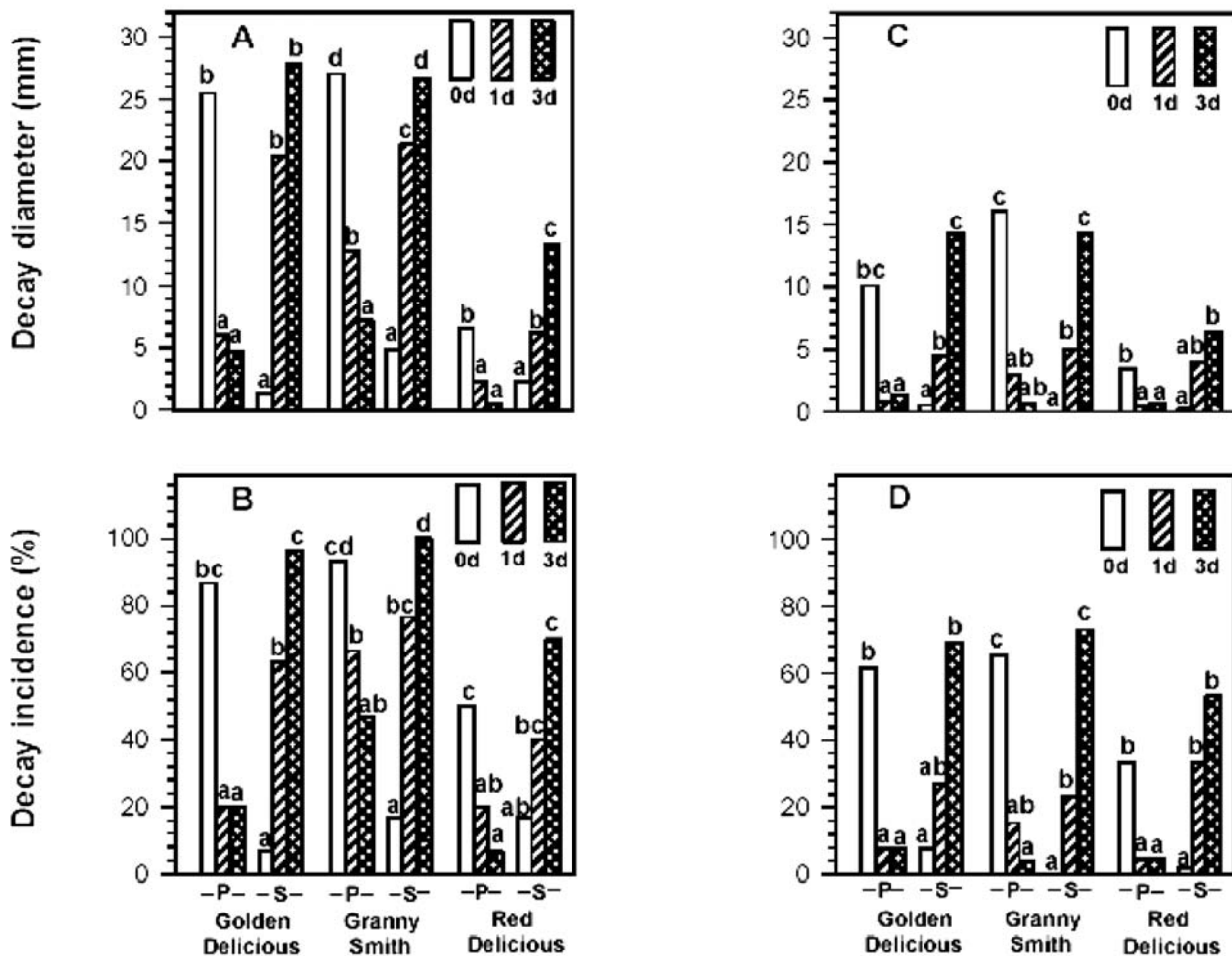
Fig. 2. Decay diameter and incidence in puncture wounds (P) compared to slice wounds (S) in fruits of apple cultivars that were inoculated with 50 conidia per wound of *Botrytis cinerea* (A and B) or *Penicillium expansum* (C and D) after incubation at 22°C for seven days. Data from two experiments were combined for analysis of variance on ranks. Values are the means of 30 replicates for *B. cinerea* and 27 replicates for *P. expansum*. In each graph (A-D) the letters that denote mean separation using the Student Newman Keul's Test are used to compare means for puncture wounds to means for slice wounds only for the stated cultivar. Within each cultivar, means accompanied by the same letter were not significantly different ( $P \leq 0.05$ ).



**Fig. 3.** Slices of apple fruits showing decay from puncture (P) or slice (S) wounds that had been inoculated with 50 conidia per wound of *Botrytis cinerea* in freshly-made wounds [0 day (d)], or in wounds that were 1d or 3d-old prior to inoculation. Representative results showing the general wound-type effect after incubation at 22°C for seven days.

Watkins and Rosenberger, 2000). The effect also was observed in fruits incubated for 30 days at 3°C, which are conditions that simulate post-harvest storage practices (Watkins, 2003). The effect was found in fruits that received no anti-pathogen chemical treatments prior to CA storage and in fruits that had been treated with TBZ and DPA. Moreover, fresh slice wounds were resistant to decay compared to older slice wounds, regardless if they were in fruits prior-treated with DPA and TBZ or in fruits that were not.

Results of this study show for the first time that wound type in an apple fruit affects the resistance of the wound to decay-causing fungal pathogens. Further study is needed to determine if the effect is exhibited in other apple cultivars and from challenge by other pathogens. Other pome fruits should be examined for the effect. The wound-type effect suggests that our current thinking of how wound healing relates to fungal resistance in apple fruits needs further study. Exploring



**Fig. 4.** Decay diameter and incidence in puncture wounds (P) compared to slice wounds (S) in fruits of apple cultivars that were inoculated with 500 conidia per wound of *Botrytis cinerea* (A and B) or *Penicillium expansum* (C and D) after incubation at 22°C for seven days. Data from two experiments were combined for analysis of variance on ranks. Values are the means of 25 replicates for *B. cinerea* or *P. expansum*. In each graph (A-D) the letters that denote mean separation using the Student Newman Keul's Test are used to compare means for puncture wounds to means for slice wounds only for the stated cultivar. Within each cultivar, means accompanied by the same letter were not significantly different ( $P \leq 0.05$ ).

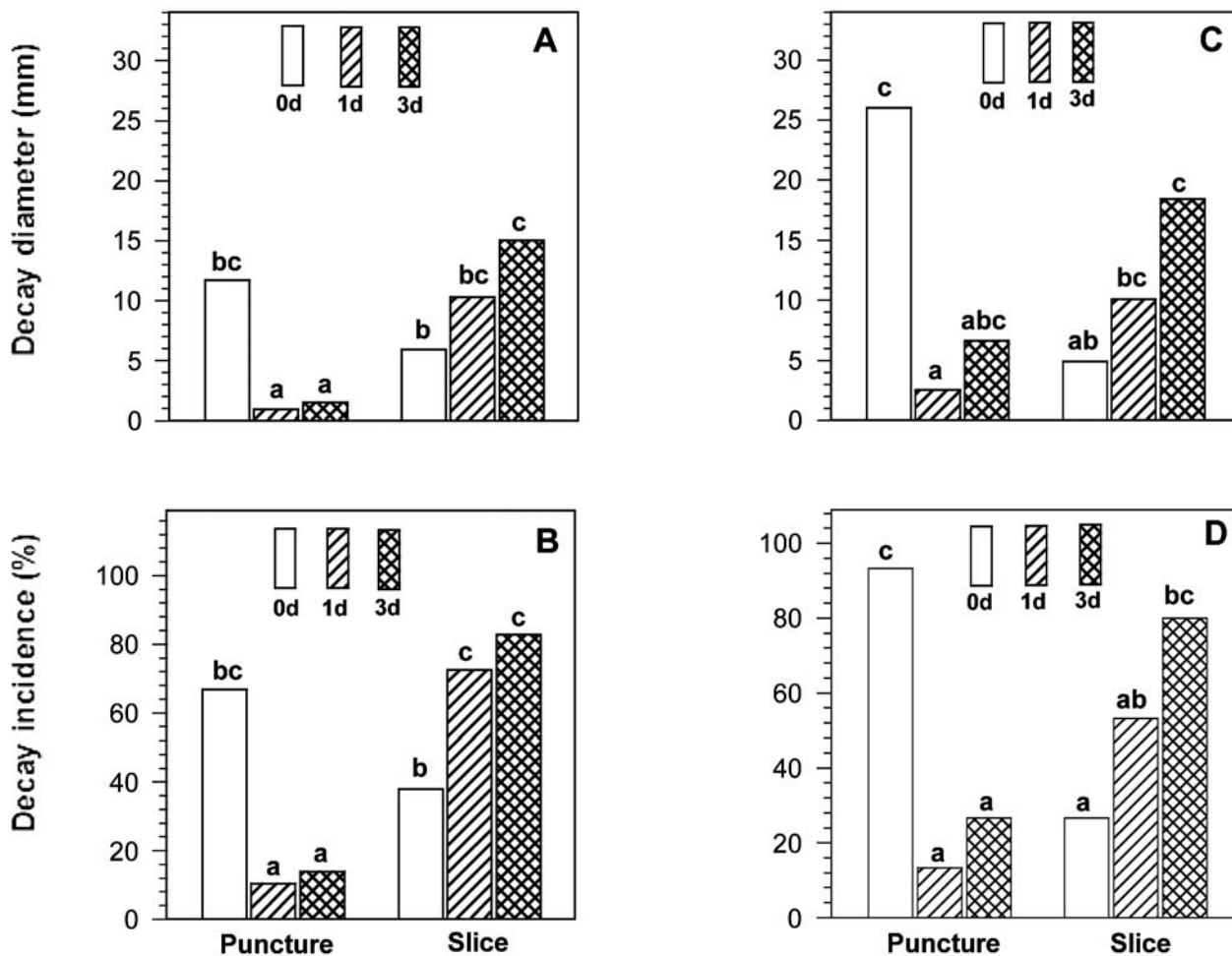


Fig. 5. Decay diameter and incidence in puncture wounds (P) compared to slice wounds (S) in Golden Delicious fruits that were inoculated with 500 conidia per wound of *Botrytis cinerea* (A and B) or *Penicillium expansum* (C and D) after incubation at 3°C for 30 days. Data from two experiments were combined for analysis of variance on ranks. Values are the means of 30 replicates for *B. cinerea* or *P. expansum*. Means accompanied by the same letter were not significantly different ( $P \leq 0.05$ ) using the Student Newman Keul's Test.

the cause of the wound-type effect may help us to better understand wound response to fungal pathogen attack in apple fruits and perhaps in other plants.

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