Increasing Celery Resistance to Pathogens during Storage and Reducing High-risk Psoralen Concentration by Treatment with GA₃

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Abstract. Since psoralens have a very weak antifungal activity in vitro, we propose that (+)marmesin, the precursor of psoralens in celery (Apium graveolens L.) is associated with celery resistance to pathogens. (+)Marmesin has at least 100 times greater antifungal activity in vitro than psoralens. After 1 month of storage at 2°C, the concentration of total psoralens increased from 8 to 67 µg·g⁻¹ fresh weight, (+)marmesin decreased from 27 to 4 µg·g⁻¹ fresh weight, and the incidence of decay increased from 0% to 34%. However, when celery was treated with GA before 1 month of storage at 2°C, decay increased to only 7%, the concentration of psoralens increased to 31 µg·g⁻¹ fresh weight and the concentration of (+)marmesin decreased to 13 µg·g⁻¹ fresh weight. It seems that GA retarded celery decay during storage by slowing down the conversion of (+)marmesin to psoralens, thereby increasing the resistance to pathogens during storage.

Linear furanocoumarins (psoralens) are believed to be phytoalexins associated with celery resistance to pathogens (Beier and Oertli, 1983; Beier et al., 1983; Chaudhary et al., 1985). Beier and Oertli (1983) demonstrated that the phytoalexin response was also initiated by general elicitors including copper sulfate, UV light, and low temperatures. Mechanical damage occurring during harvesting and storage has also been shown to increase furanocoumarin concentration from about 2 to 95 µg·g⁻¹ fresh weight (Chaudhary et al., 1985).

Psoralens produced by celery have several biological effects, the most important of which are photosensitized reactions with nucleic acids. Photosensitized reactions of DNA with furanocoumarins cause lethal, mutagenic, and clastogenic effects in a variety of cellular systems (Ashwood-Smith et al., 1980, 1982; Scott et al., 1976). Photosensitization occurs in animals exposed to the combined effects of psoralens and near-UV light; it probably also occurs in humans. Effective wavelengths for these phototoxic reactions are 320–380 nm (Chaudhary et al., 1985; Igali et al., 1970; Miller et al., 1984). Psoralen-UV light (PUVA) phototherapy for psoriasis is recognized by the World Health Organization to be causally related to human skin cancer (IARC, 1983).

Phytophotodermatitis (a skin disorder caused by psoralens) has been reported in field workers after contact with celery (Austad and Kalvi, 1983; Birmingham et al., 1961; Finkelstein et al., 1994). Other studies (Berkley et al., 1986; Seligman et al., 1987) have found that phytophotodermatitis occurs in grocery workers. Psoralens also exist in other plants, such as parsnips, parsley, figs, and citrus (Pathak et al., 1961).

Gibberellins (GA), are commonly occurring phytohormones that exist in relatively high content in juvenile plant tissues compared with older tissues (Nooden, 1988). GA activity has been found to delay senescence and reduce leaf senescence (Aharoni and Richmond, 1978; Chin and Beever, 1970; Nooden, 1988). This trend is accompanied by decreased resistance of the plant tissue to microorganisms associated with celery resistance to pathogens (Beier and Oertli, 1983; Beier et al., 1983; Chaudhary et al., 1985). Beier and Oertli (1983) demonstrated that the phytoalexin response was also initiated by general elicitors including copper sulfate, UV light, and low temperatures. Mechanical damage occurring during harvesting and storage has also been shown to increase furanocoumarin concentration from about 2 to 95 µg·g⁻¹ fresh weight (Chaudhary et al., 1985).

Materials and Methods

Fungal, plant material and GA treatment. The fungi Botrytis cinerea Pers., Alternaria alternata (Fr.) Keissler, and Sclerotinia sclerotiorum (Lib.) Dby were used in the experiments. These fungi, which are major pathogens causing rots diseases to celery, were isolated from naturally infected ‘Tender Crisp’ celery from Kibbutz Alumim, the Negev Desert, Israel, in January 1991. ‘Early Bell’ celery petioles were used for the experiments and inoculations. Percentages of decay before or during leaf senescence (Aharoni and Richmond, 1978; Chin and Beever, 1970; Nooden, 1988). This trend is accompanied by decreased resistance of the plant tissue to microorganisms associated with celery resistance to pathogens (Beier and Oertli, 1983; Beier et al., 1983; Chaudhary et al., 1985). Beier and Oertli (1983) demonstrated that the phytoalexin response was also initiated by general elicitors including copper sulfate, UV light, and low temperatures. Mechanical damage occurring during harvesting and storage has also been shown to increase furanocoumarin concentration from about 2 to 95 µg·g⁻¹ fresh weight (Chaudhary et al., 1985).

(+)-Marmesin has been reported as the precursor of linear furanocoumarins in several species belonging to the Apiaceae, Rutaceae, Moraceae, and Leguminosae (Brown, 1978; Floss, 1972; Floss and Mothes, 1966; Floss and Paikert, 1969; Hamerski and Matern, 1988; Matern et al., 1988; Steck et al., 1969). The main objectives of this work are to determine whether (+)-marmesin, rather than psoralens, may play the major role in celery resistance to pathogens during storage, and whether GA increases celery resistance to pathogens by slowing down the conversion of (+)-marmesin to psoralens and maintaining high levels of (+)-marmesin and low levels of psoralens in celery.

Received for publication 6 Oct. 1994. Accepted for publication 10 Feb. 1995. Contribution from the Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel. No. 1320-E, 1995 series. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked advertisement solely to indicate this fact.

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562 J. Amer. Soc. Hort. Sci. 120(4):562–565. 1995.
petioles inoculated with *B. cinerea*. Four to five 3-mm-long incisions, 0.2-0.5 mm deep, were cut with a sterile scalpel into each celery petiole (5–30 cm long and 2–3 cm thick). A 3-mm-diameter disk, cut from an actively growing potato-dextrose agar (PDA) culture of *B. cinerea*, was placed over each incision, fungal side downward, and the inoculated petioles were incubated for 4 days in a dark chamber at 98% relative humidity and 24°C.

*Extraction, purification, isolation, identification and quantification of psoralens and (+)marmesin in celery.* Extraction was done 4 days after inoculation with *B. cinerea*. Slices of inoculated celery petioles with necrotic lesions cut from the margins of the wounds (220 g fresh weight) were extracted with distilled water, at 10 ml·g fresh weight tissue, for 2 h at 40°C. Following partition with ethyl acetate (EtOAc) and concentration by evaporation of the solvent at 40°C in a Rotovac evaporator, purification, isolation, and identification of psoralen, bergapten, xanthotoxin, and isopimpinellin were performed using known techniques (Beier, 1985; Beier et al., 1983b; Dercks et al., 1990; Trumble et al., 1990). The crude material (340 mg) was loaded onto a vacuum column (Merck Silica H, 5 g, packed into a 2-cm-i.d., 30-cm-tall, sintered glass funnel, evacuated by a water aspirator) and eluted in petroleum ether with an EtOAc increasing step gradient to yield 12 fractions (50 ml each). The fractions were assayed for antifungal activity against *B. cinerea*. Fraction 7, eluted from the column with 40% EtOAc in petroleum ether, was the most active against this fungus. Preparative high-performance liquid chromatography (HPLC) separations were performed with an Applied Biosystems instrument equipped with two model 150 pumps and an 893 program- ment equipped with two model 150 pumps and an 893 program-

library and were identified by comparison with the NIST database and were identified by comparison with the NIST database.

The chromatogram yielded eight components, with relative retention times (t_R) of 7.5, 8.5, 9.3, 11.5, 13, 14, 14.8, and 18.1 min. The last component (t_R = 18.1 min, 3.1 mg) was found to be the main active compound of the mixture. Mass spectra were recorded on a Finnigan MAT ITD-800 GC-MS instrument equipped with a DB-1 capillary, minibore column (carrier gas: helium, 1 ml·min) coupled to the NIST mass-spectra library and were identified by comparison with the NIST database as (+)marmesin. GC-MS data (t_R = 21.13 min, 70eV) m/z 246 (M+, 100%), 213, 187, 160, 131, 102, 77, and 59. NMR spectra were recorded on a Bruker WM-360 operating at 360.132 MHz for CDCl3 (Cary, N. C.) package. Experiments were conducted three times.

**Results and Discussion**

Our findings indicate that (+)marmesin, and not psoralens, is the major compound involved in celery resistance to pathogens. In vitro, EC_50 values of (+)marmesin has at least 100 times greater antifungal activity than psoralens (Table 1). There was no effect of the light on antifungal activity of psoralens and (+)marmesin. The in vivo concentration of psoralens in celery is 8 µg·g fresh weight.

Table 1. Effective concentrations of psoralens and their precursor, (+)marmesin, needed for obtaining 50% growth inhibition (EC_50) of three pathogenic fungi of celery.

| Pathogens                  | Psoralen | Bergapten | Xanthotoxin | Isopimpinellin | (+)Marmesin |
|----------------------------|----------|-----------|-------------|----------------|-------------|
| *Botrytis cinerea*         | 4150     | 4740      | 4550        | 5270           | 28          |
| *Alternaria alternata*     | 5040     | 5890      | 5540        | 6330           | 43          |
| *Sclerotinia sclerotiorum* | 3620     | 3970      | 3310        | 4050           | 19          |
and this is only 0.27% of the concentration required for growth inhibition of celery pathogens in vitro. On the other hand, the EC₅₀ values of (+)marmesin (19, 28, and 43 µg·ml⁻¹) is close to the natural amount occurring in vivo (27 µg·g⁻¹ fresh weight). Additionally, increased susceptibility of stored celery to pathogens was accompanied by a decrease in (+)marmesin concentration and a corresponding increase in total psoralen concentration (Fig. 1). An increase in celery decay has been found to be negatively correlated with (+)marmesin concentration (r² = 0.927; y = 33.2 – 0.28x) and positively correlated with psoralen concentration (r² = 0.956; y = 8.3 + 0.47x). After 1 month of storage at 2°C, the concentration of total psoralens (psoralen, bergapten, xanthotoxin, and isopimpinellin) increased from 8 to 67 µg·g⁻¹ fresh weight, whereas the concentration of (+)marmesin under the same storage conditions decreased from 27 to 4 µg·g⁻¹ fresh weight. Incidence of decay after 1 month of storage at 2°C was 34% (Fig. 1). All of these findings indicate that psoralens cannot play a major role in the defense mechanism of celery against these pathogens and that (+)marmesin is the major compound involved. In support of our findings, two other research groups recently reported that psoralens may not play a major role in disease and insect resistance of celery (Diawara et al., 1992, 1993; Heath-Pagliuso et al., 1992).

The conversion of (+)marmesin to the respective linear furanocoumarins involves the oxidative cleavage of the isopropyl alcohol sidechain and the introduction of a double bond to the five-member ring moiety (Brown, 1978; Hamerski and Matem, 1988; Matem et al., 1992). We consider, therefore, that following this reaction the resultant products (psoralens), derived from (+)marmesin, lose about 99% of their antifungal activity.

Several studies (Aharoni and Richmond, 1978; Aharoni et al., 1975; Barkai-Golan and Aharoni, 1981; Nooden, 1988; Shaul et al., 1992) reported that treatment with the phytohormone GA₃ decreased plant decay caused by pathogens, but the mechanism of GA₃ action in increasing plant resistance remained unclear. Similarly, the results of the present study show that celery stored after treatment with GA₃ was more resistance to pathogens than the nontreated control.

Treatment of celery with GA₃ before storage for 1 month at 2°C resulted in 7% decay only. Concentrations of total psoralens and (+)marmesin after 1 month of storage at 2°C following treatment with GA₃, were 31 and 13 µg·g⁻¹ fresh weight, respectively (Fig. 1). GA₃ does not have any effect on the growth of fungi and bacteria in vitro.

We suspect, therefore, that GA₃ retarded celery decay during storage, by slowing down the conversion of (+)marmesin to psoralens, thereby maintaining a high level of (+)marmesin and low levels of psoralens and, by this, increased celery resistance to pathogens during storage. This, in turn, may suggest a possible mechanism by which GA₃ reduces celery decay during storage.

A routine postharvest treatment of celery with GA₃ would achieve a double goal: decreasing psoralen concentration and contributing to consumer health and increasing the disease resistance in celery as an alternative to the postharvest application of pesticides, the use of which is limited in many countries.

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Fig. 1. Concentration of total psoralens (A) and (+)marmesin (B), and incidence of decay (C) in celery during 4 weeks of storage at 2°C, treated and nontreated with GA₃. Vertical bars indicate standard error.
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