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INFLUENCE OF EXOGENOUS ETHYLENE ON IPOMEAMARONE ACCUMULATION IN BLACK ROT INFECTED SWEET POTATO ROOTS

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Abstract—Ipomeamarone accumulation in sweet potato (*Ipomoea batatas*) roots infected with *Ceratocystis fimbriata* (black rot) was decreased by one-third when roots were stored under 100 ppm ethylene. This effect of ethylene was not observed when infected tissue was also treated with benzylisothiocyanate. Ethylene treatment and long term infection were associated with the accumulation of 4-ipomeanol and 1-ipomeanol.

INTRODUCTION

Ethylene may play an important role in host-parasite interactions as indicated by studies with banana wilt disease and black rot in sweet potato [2]. It is known that infection of sweet potato roots with *Ceratocystits fimbriata* results in a high rate of ethylene production lasting several days [3–6]. Stahmann *et al.* [2] showed that, upon exposure to ethylene, root tissue from a *C. fimbriata* susceptible variety of sweet potato developed resistance to the pathogen. In addition, the capacity of non pathogenic isolates of fungi to induce host resistance to pathogens is correlated positively with their capacity to accumulate ethylene. Alternatively, Chalutz and Devay [4] reported that ethylene applied exogenously to sweet potato roots before or after infection did not affect black rot development.

Ipomeamarone and related furanoterpenoids accumulate in sweet potato roots which are mold-damaged or otherwise traumatized [6–8]. Black rot resistant varieties of sweet potato root accumulate ipomeamarone more rapidly in response to infection than susceptible varieties [6].

Other studies [9] indicate that ipomeamarone and related compounds which accumulate in sweet potato roots are toxic to mammals and that they are present in potatoes selected from retail and wholesale market channels. In the light of the findings that ethylene gas affects black rot resistance of sweet potato roots we have examined the influence of this gas on ipomeamarone accumulation.

RESULTS AND DISCUSSION

Root plugs infected with *C. fimbriata* accumulated detectable levels of ipomeamarone after 48 hr infection. Ipomeamarone levels were highest after *ca* 120 hr infection and subsequently decreased. Mycelium growth was evident at 48 hr and the necrotic ring, characteristic of black rot, was clearly discernible at 96 hr after infection. There was no obvious difference in the mold growth on infected samples treated with ethylene or benzylisothiocyanate (BI) in comparison to those stored in air. At 144 hr, the air stored samples had somewhat larger necrotic rings (3–4 mm) than the ethylene treated roots (2–3 mm). Mold growth was checked in all infected samples coincident with the appearance of the necrotic ring. About 3-fold higher levels of ipomeamarone accumulated in samples stored under air than in samples stored under air containing 100 ppm ethylene (Fig. 1). Roots incubated in air treated or untreated with BI had comparable levels of ipomeamarone. However, roots incubated in ethylene and treated with BI accumulated substantially more ipomeamarone than the infected samples (no BI treatment) under ethylene. The results indicate that high levels of ethylene lead to depressed levels of ipomeamarone accumulation and further, that BI can reverse the apparant inhibitory action of ethylene treatment. Patel and Tang [10] have demonstrated that BI is an endogenous inhibitor of ethylene biogenesis in papaya fruit. We suggest that the ability of BI to overcome the action of exogenous ethylene on ipomeamarone accumulation relates to the suppression of an autocatalytic ethylene generating system of the sweet potato root. It is clear that samples not treated with ethylene also synthesize the gas, although ethylene concentrations were probably less than 1 ppm in our experiment [4]. It is possible that low levels of ethylene stimulate ipomeamarone biogenesis while high levels of the gas depress the accumulation of this metabolite.

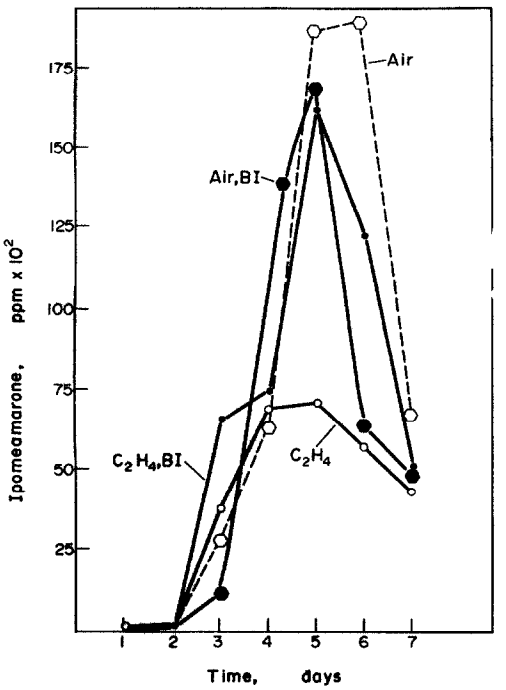

Fig. 1. Ipomeamarone content of sweet potato root plugs after infection with *C. fimbriata*. Roots were stored in air (○), 100 ppm ethylene in air (○), treated with benzylisothiocyanate and stored in air (●) or treated with benzylisothiocyanate and stored in air containing 100 ppm ethylene (●). Data are duplicate analyses for one experiment and are representative of results obtained in a separate experiment. Mechanically wounded plugs (not infected with *C. fimbriata*) did not accumulate appreciable quantities of ipomeamarone.

TLC revealed the presence of 3 major Erhlich positive components in addition to ipomeamarone. The zone with R_f 0·46 has been identified as a mixture of 1-ipomeanol, and 4-ipomeanol [11]. The intensity of the C_9 terpene zone was greatest in ethylene treated samples and increased markedly after 120 hr in roots incubated in air. This observation leads us to suggest that ethylene gas acts by shunting ipomeamarone or an intermediate of ipomeamarone to 4-ipomeanol and 1-ipomeanol. Mechanically wounded plugs of tissue did not accumulate appreciable levels of ipomeamarone (less 100 ppm) or other Ehrlich positive components after 7 days.

EXPERIMENTAL

Sweet Potatoes (*Ipomea batatas*, cv Centennial) were harvested, dry cured and stored at 15° for *ca* 6 months prior to expts. Spore cultures of *Ceratocystis fimbriata* were obtained from black rot lesions on sweet potatoes and cultured on potato-dextrose agar. Roots were washed with 0·05% NaClO prior to inoculation. Cylindrical plugs of tissue (1 × 3 cm) were removed from roots with a borer and soaked with either *C. fimbriata* spores suspended in dist H_2O, with spores, 0·2 M BI, or 0·2 M BI with spores. The treated plugs were re-inserted into the root. Treated and intact roots were placed in 10 l. jars fitted with valved tops. Jars were flushed at 12 hr intervals with either air or air containing 100 ppm C_2H_4. Jars were incubated at 22–24° and the O_2 concn of the headspace was routinely assayed. The O_2 concn was never lower than 18% after 12 hr.

Plugs were removed at 24 hr intervals over 7 days, weighed and refluxed with 10 vol $CHCl_3$ for 30 min. Extracts were cooled to room temp, centrifuged (2100 *g*, 5 min) and stored under N_2 at −12° prior to analysis for terpenes. Samples were spotted on Si gel TLC on plates and developed in C_6H_6–MeOH–methanol (10:1). The plates were sprayed with Ehrlich's reagent (5 g dimethylaminobenzaldehyde in 20 ml conc HCl and 80 ml 95% EtOH). Ipomeamarone has an R_f of 0·6 and a characteristic pink color which fades to gray-black. 4-ipomeanol has an R_f of 0·47 and a characteristic pink-purple color after treatment with Ehrlich's reagent [11].

Ipomeamarone was quantitatively analyzed by GLC using a FID instrument and a 2 m × 6 mm glass column packed with 10% SE 30. The column temp was 180° and the carrier gas was He. GLC operations were similar to those given in ref [9].

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