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Imazalil Enhances the Shoot-Inducing Effect of Benzyladenine in *Spathiphyllum floribundum* Schott

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Abstract. In *Spathiphyllum floribundum* 'Petite' Schott shoot induction by benzyladenine (BA) was enhanced dramatically by adding the imidazole fungicide imazalil to the medium. As the concentration of imazalil increased, the number of shoots increased, and finally their size was reduced to a small meristematic dome. An average of 127 shoots/explant developed when 2.5 mg/L BA was combined with 16 mg/L imazalil. Doubling the BA concentration had no significant effect on shoot induction. Imazalil did not affect the root-inhibiting effect of BA. When imazalil was applied without BA, the number of roots and total root length/plant were reduced, but no new shoots developed.

Imazalil is an imidazole fungicide (Fig. 1). The fungicidal action of imidazoles, triazoles, and pyrimidine-carbinoles is based on inhibition of ergosterole biosynthesis. Several representatives of the aforementioned groups such as ancymidol and paclobutrazol are known as growth retardants in plants, where they inhibit the oxidative reactions leading from *ent*-kaurene to *ent*-kaurenoic acid and thus inhibit GA biosynthesis. Although imazalil is not a growth retardant, it shares a structural feature common to all of these compounds: a heterocyclic ring containing a sp^2-hybridized nitrogen with a lone electron pair. Their target enzymes are monooxygenases (Rademacher 1991), so they might affect several other biochemical pathways. This article reports the remarkable synergistic action of imazalil and BA on adventitious shoot formation of in vitro cultured *Spathiphyllum floribundum*. This important ornamental crop belongs to the Araceae and is usually micropropagated by axillary and adventitious shoots, both induced at the plant base (Fonnesbech and Fonnesbech 1979).

Abbreviations: GA, gibberellic acid; BA, benzyladenine; [9G]BA, 9-β-D-glucopyranosylbenzyladenine; [9R]BA, 9-β-D-ribofuranosylbenzyladenine.

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Materials and Methods

S. floribundum 'Petite' Schott was micropropagated in Meli-jars (De Proft et al. 1985) on a medium containing Murashige and Skoog (1962) macroelements, Nitsch and Nitsch (1969) microelements, 35 mg/L NaFe/EDTA, 100 mg/L inositol, 0.4 mg/L thiamine HCl, 3 g/L Roth agar, and 4 g/L BDH agar. In a 3 × 3 factorial experiment 0, 4, or 16 mg/L imazalil (trade name Fungaflor, LIRO, Belgium) was combined with 0, 2.5, or 5 mg/L BA. Each treatment consisted of eight replications (eight vessels). The cultures were maintained at 23 ± 2°C under a 16-h photoperiod at 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR. Each culture vessel contained 100 mL of autoclaved (120°C, 20 min) medium and was inoculated with six single shoots of which the leaf blades were removed. After 10 weeks the induced shoots were counted, as well as the roots, whose length was also measured. This experiment was repeated twice.

Results

On medium without BA and 0, 4, or 16 mg/L imazalil, roots were formed, but no shoots were induced (Tables 1–3). Increasing the imazalil concentration reduced the number of roots and the total root length/plant. BA without imazalil gave a normal multiplication rate. When 2.5 mg/L BA was combined with 4 mg/L imazalil, the basal part of the explants started to swell after a few weeks. Then a callus-like structure appeared, on which a ring of numerous small shoots developed. After 10 weeks this resulted in a globe-shaped cluster of shoots or shoot primordia. It was clear that these shoots had an adventitious origin. When 2.5 mg/L BA was combined with 16 mg/L imazalil, the number of shoots increased even more, but their size was reduced to a small meristematic dome (Fig. 2). Notwithstanding the concentration of imazalil, doubling

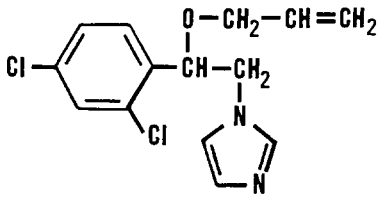

Fig. 1. Structure of imazalil.

Table 1. Interaction of BA with imazalil in *Spathiphyllum:* number of induced shoots/plant after a culture period of 10 weeks. Significant differences (L.S.D. 95%) are indicated with different letters.

Imazalil (mg/L)	BA (mg/L) 0	2.5	5
0	0 a	28 b	33 b
4	0 a	73 c	79 c
16	0 a	127 d	157 d

the BA concentration only reduced the number of roots and the total root length.

Discussion

In an attempt to eliminate fungi from plant tissue cultures Shields et al. (1984) applied imazalil in the medium of protoplasts, root cultures, and callus cultures of *Nicotiana tabacum*. They concluded that imazalil has a fairly broad antifungal spectrum and has relatively few phytotoxic effects. They noticed a deformed thickening of lateral roots.

More is known about the effect of paclobutrazol in liquid cultures. Paclobutrazol is not an imidazole but has also a heterocyclic ring containing a sp^2-hybridized nitrogen with a lone electron pair (Rademacher 1991). Paclobutrazol and to a lesser extent ancymidol inhibited leaf development and induced the formation of bud clusters in liquid-cultured *Gladiolus* (Ziv 1990) and the Araceae *Philodendron* (Ziv and Ariel 1991). It is interesting that the growth retardants were always combined with BA. Although it is difficult to compare these experiments with out work, paclobutrazol seems to have effects on bud proliferation similar to those of imazalil.

We demonstrated that imazalil enhanced greatly the shoot-inducing effect of BA in *Spathiphyllum*. The number of shoots was not influenced regardless of whether 2.5 or 5 mg/L was used. However, BA had to be present. Without BA, imazalil did not induce shoots. Without BA, increasing the concentration of this fungicide only reduced root induction and root elongation. When combined with BA, increasing imazalil concentrations no longer affected the roots; then only BA was responsible for inhibiting root induction and elongation.

Table 2. Interaction of BA with imazalil in *Spathiphyllum:* number of roots/plant after a culture period of 10 weeks. Significant differences (L.S.D. 95%) are indicated with different letters.

Imazalil (mg/L)	BA (mg/L) 0	2.5	5
0	3.61 d	3.63 d	2.10 abc
4	2.43 bc	3.45 c	1.52 ab
16	1.42 a	3.30 c	1.55 ab

Table 3. Interaction of BA with imazalil in *Spathiphyllum:* total root length/plant (cm) after a culture period of 10 weeks. Significant differences (L.S.D. 95%) are indicated with different letters.

Imazalil (mg/L)	BA (mg/L) 0	2.5	5
0	14.7 c	8.2 b	1.7 a
4	6.9 b	8.2 b	1.0 a
16	1.1 a	6.4 b	2.7 a

Fig. 2. *S. floribundum* 'Petite.' The shoot-inducing effect of BA is enhanced by imazalil. Left, subcultured on 5 mg/L BA; right, subcultured on 5 mg/L BA + 16 mg/L imazalil.

The mode of action of imazalil is unclear; it only induces shoots in the presence of exogenous cytokinins such as BA. This indicates that imazalil might alter BA metabolism or catabolism. When cultured on a medium containing BA, *Spathiphyllum* mainly converts BA into large amounts of [9G]BA, which is considered a storage or a detoxification product, and into [9R]BA, which still has cytokinin activity (Werbrouck et al. 1995). Imazalil might influence the concentration of the active BA

metabolites. It could also have an inhibitory effect on their catabolic enzymes. However, the results suggest that imazalil has no effect on the conjugation or catabolism (cytokinin oxidase) of the endogenous cytokinins of *Spathiphyllum;* when imazalil is applied without BA, no new shoots are induced. In these combinations only root initiation and elongation are affected, which could be a result of auxin metabolism. Probably imazalil has more than one mode of action and can offer a tool for studying the metabolism of cytokinins as well as auxins.

Although an interaction with the BA metabolism is more likely, an action on GA metabolism cannot be excluded because of the parallelism in structure between imazalil and growth retardants.

We examined the combined effect of imazalil and BA on other micropropagated species. *Rosa* hybrids and *Ficus benjamina* gave no higher multiplication rates, whereas *Anthurium scherzerianum* showed a response that was weaker but similar to that of *Spathiphyllum*. It is possible that the reported action of imazalil is restricted to monocots or even to a limited number of monocot families.

Conclusions

In *S. floribundum* 'Petite' imazalil enhances the shoot-inducing effect of BA without affecting its root-inhibiting effect. Probably imazalil influences the metabolism or catabolism of BA in this crop.

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