Does IBA Inhibit Shoot Growth in Rooted Cuttings?

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Single-node 'Royalty' rose cuttings were utilized to examine the relationship between adventitious root formation, bud break, and ethylene synthesis of cuttings following IBA treatment. IBA application increased rooting and inhibited the bud break of cuttings. IBA≥600 mg·liter^{-1} almost completely inhibited bud break of cuttings during four weeks of rooting. IBA treatment stimulated ethylene synthesis, which was inversely correlated with bud break of cuttings. Ethephon also significantly inhibited bud break. Bud break of rose cuttings was completely prevented by repeated ethephon sprays used to maintain high endogenous ethylene levels during the first 10 days. Treatment with STS, and ethylene action inhibitor, improved bud break.

REVIEW OF LITERATURE

Synthetic auxins, such as indole-3-butyric acid (IBA) and naphthaleneacetic acid (NAA), are commercially used to promote adventitious root formation of cuttings. They are more effective than indole-3-acetic acid (IAA) because of their greater stability within tissue and during storage (Hartmann et al., 1990). However, application of synthetic auxins to stem cuttings was found to inhibit bud development of cuttings in several species (Christense et al., 1980; DeVries and Dubois, 1988). Auxins applied at high concentrations even prevent shoot growth or shoot abscission after bud break, although root formation was adequate (Hartmann, 1990). For example, IBA at 1000 to 2500 mg·liter^{-1} caused almost complete bud abscission of 'Ennis' and 'Cassina' hazelnut softwood cuttings (Bassil et al., 1991).

After rooting, the buds of cuttings of many species enter a period of dormancy before they are able to resume shoot growth (Goodman and Stimart, 1987; Hartmann et al., 1990; Smalley and Dirr, 1986). Early bud break and shoot growth are considered important factors regulating the overwinter survival of newly propagated cuttings of *Acer, Cornus, Hamamelis, Magnolia, Prunus, Rhododendron* and *Viburnum* (Goodman and Stimart, 1987; Smalley and Dirr, 1986). Many actions of auxin are mediated by the synthesis of ethylene (Burg and Burg, 1968). Our hypothesis concerning the auxin inhibition of bud break in cuttings is that auxins applied to cutting bases increase ethylene synthesis in the upper part of the cutting, and as a result of the high endogenous ethylene concentration, bud break of cuttings is inhibited or bud dormancy is induced. In the present study, we used single-node 'Royalty' rose cuttings as a model system.

MATERIALS AND METHODS

Five to six-year-old stock plants of 'Royalty' rose were grown in benches or containers in a medium of 1 perlite : 1 peat : 1 soil (by volume). The greenhouse was at 21/16°C (day/night) in spring and winter, and with 16 h photoperiod achieved by high intensity discharge lamps hanging 2 m apart and 1.5 m above plants. Fertilization with 20N-20P-20K was applied weekly at 200 mg·liter^{-1}. Rose shoots

were excised for use when flower buds grew to 1.5 to 2.0 cm in diameter. Single-node cuttings with 4 leaflets were taken only from node 4 to 8 (distal to proximal) in order to obtain uniform cutting materials. For STS application, entire cuttings were held 20 min in a 0.5 mM (Ag+) STS solution prepared according to Reid et al, (1980) and then washed with tap water to remove superficial STS residue. Ethephon was applied at 300 to 500 mg·liter^{-1} by foliar spray until runoff.

Cuttings were rooted in a medium of 3 perlite : 2 peat moss (by volume) under intermittent mist operated for 5 sec every 4 min from 6:00 a.m. to 10:00 p.m. Temperature of the rooting medium was 20 to 23°C in spring and winter. Percent bud break of cuttings was recorded at intervals from 2 to 5 days. A lateral bud 0.7 cm in length was counted as broken. Cuttings were harvested after 20 to 30 days. All cuttings with roots ≥ 1 mm were considered as rooted.

For endogenous ethylene determination, three samples of four cuttings were randomly taken at each sampling date, except for one experiment in which eight cuttings were used. Ethylene gas in cuttings was extracted by placing cuttings in de-gassed water in a vacuum desiccator and reducing air pressure to a range of 91 to 95 Kpa (50 to 80 mm Hg height) for 4 min. An inverted funnel sealed with a rubber stopper was placed over the cuttings to collect gas bubbles. With this method, 1 to 1.5 ml gas could be collected from four cuttings and 0.8 to 1.0 ml was injected for gas chromatographic analysis. To avoid the interference of stress-induced ethylene due to sampling disturbances, all samples taken at the same time were completed within 45 to 50 min.

A completely randomized design was used in this study with 4 to 5 replications of 10 to 24 cuttings in each replication. The experiment of bud break response to IBA concentrations, however, had only two replications of 16 to 18 cuttings.

RESULTS

The number of roots per cutting increased for IBA concentrations up to 600 mg·liter^{-1} but did not increase there after (Fig. 1). IBA treatment remarkably reduced percent bud break of cuttings, even at a concentration as low as 100 mg·liter^{-1}. IBA ≥ 600 mg·liter^{-1} almost completely inhibited bud break of cuttings during the first four weeks. Percent bud break and the number of roots per cutting were negatively correlated ($r = -0.807$, $P = 0.0002$).

IBA significantly stimulated ethylene synthesis in rose cuttings. Endogenous ethylene concentration peaked after 2 or 3 days following IBA treatment (Fig. 3). During this period, ethylene concentration of cuttings treated with 500 and 1000 mg·liter^{-1} IBA was 4 and 10 times that of the control cuttings, respectively. Significant differences in ethylene production of cuttings were between three treatments still observed even after 20 days. Ethylene levels were well correlated with bud break of cuttings. The control cuttings had lowest ethylene level and highest percent bud break. IBA treatment at 1000 mg·liter^{-1} stimulated more ethylene production and more seriously delayed bud break of cuttings than did the 500 mg·liter^{-1} IBA treatment (Fig. 2).

A timely correlation was also observed between ethylene concentration and bud break of cuttings. The course of bud break for the control cuttings could be divided into three distinct periods, from 0 to 13 days, 13 to 25 days and 25 to 43 days. The rate of bud break was a constant within each period (Fig. 2). The regression-estimated slopes were 3.6, 0.8 and 2.4 for the three periods, respectively. Bud break

in the second period was significantly slower than those of the other two periods (P=0.0001). Such a rate change was preceded with a high endogenous ethylene concentration in the cuttings (Fig. 3).

When ethephon solution at 500 mg·liter^{-1} was sprayed once daily on leaves of cuttings to maintain high endogenous ethylene levels during the first 10 days of

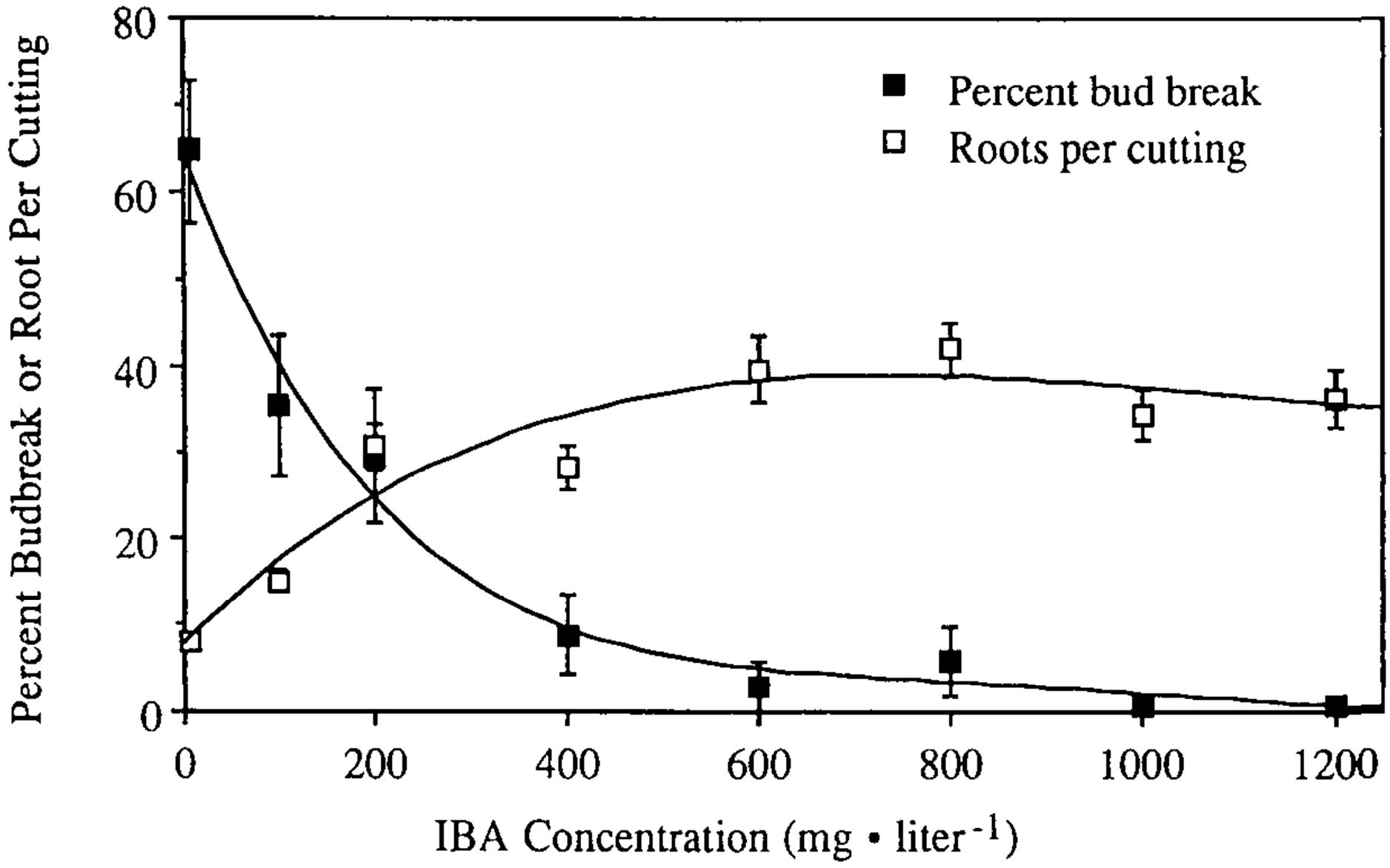

Figure 1. Rooting and bud break of 'Royalty' rose single-node cuttings following IBA treatment before rooting. Each treatment used 33 to 35 cuttings, which were harvested after 28 days. Bars denote the SE of the mean.

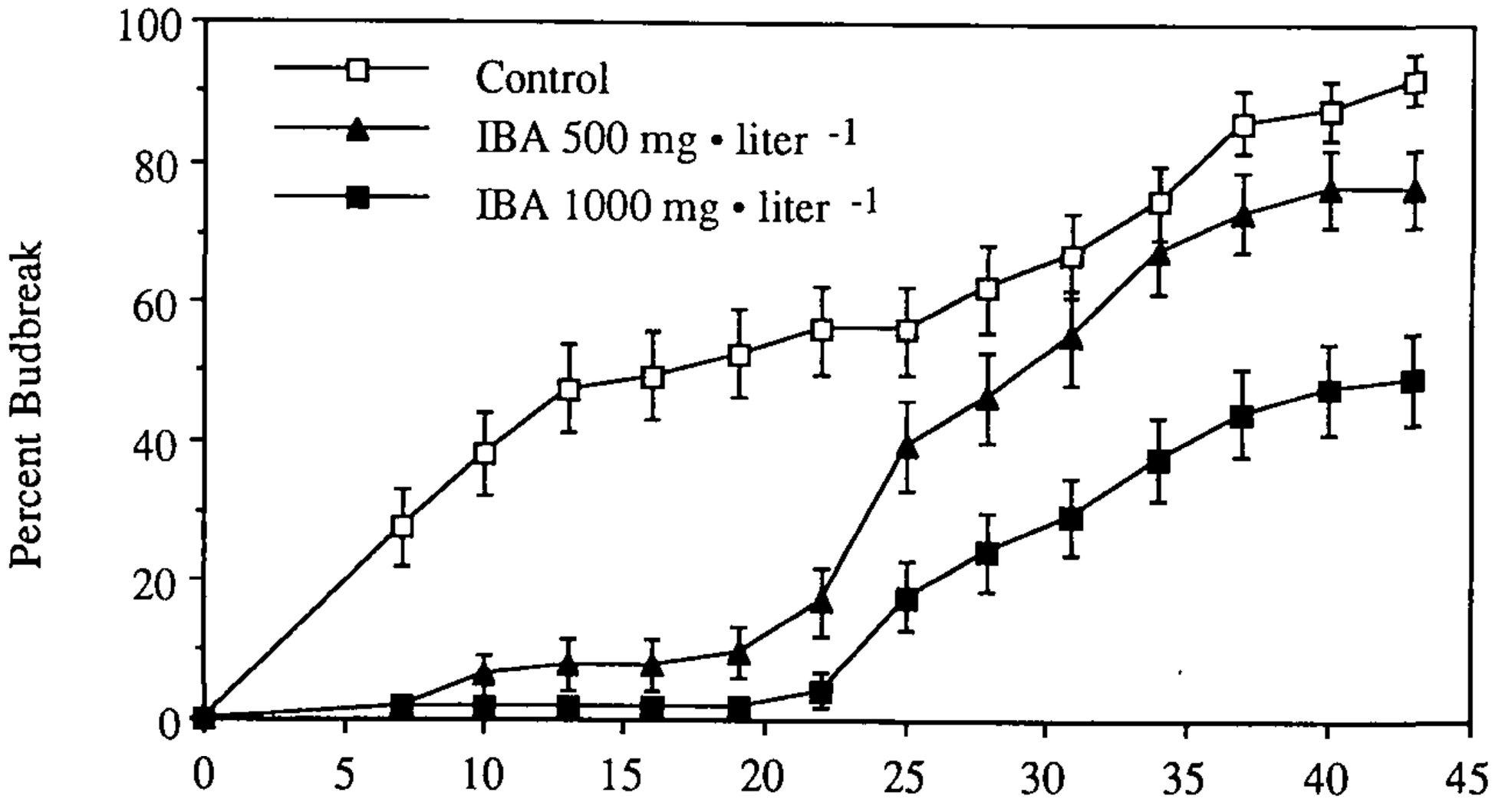

Figure 2. Bud break of 'Royalty' rose single-node cuttings following IBA treatment before rooting. Each point is the average value of four replications with 14 to 18 cuttings in one replication. Bars denote SE of the mean.

rooting, ethephon completely inhibited bud break as did the IBA treatment (Fig. 4).

More than 90% of STS-immersed cuttings broke their buds during the first 12 days of rooting, while IBA-treated cuttings showed <40% bud break even after 60 days (Fig. 5).

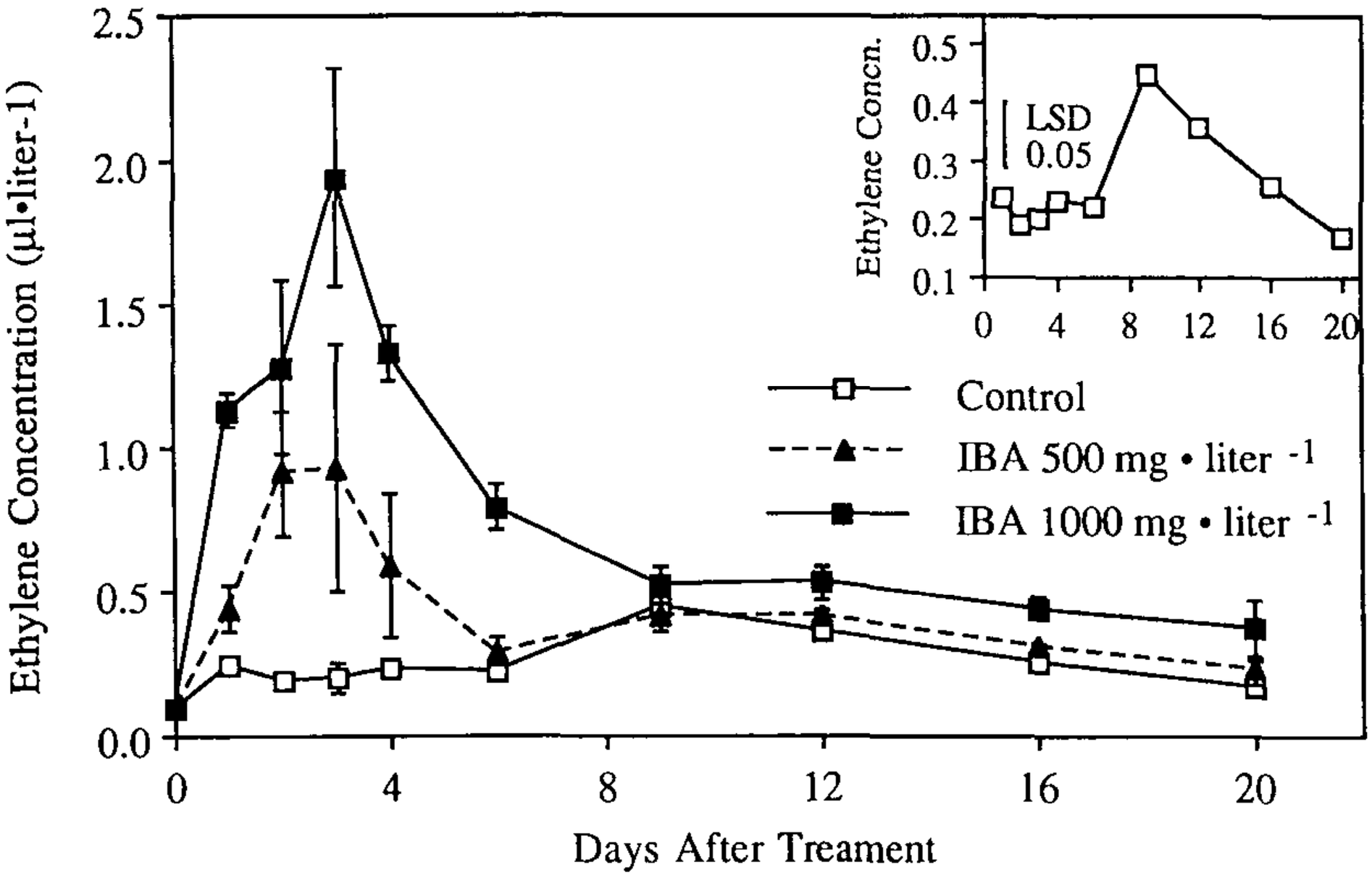

Figure 3. Endogenous ethylene concentration of 'Royalty' rose single-node cuttings following IBA treatment before rooting. The upper-right graph shows the change of ethylene concentration of the control cuttings over the time (day). Three samples of four cuttings were taken for ethylene determination. Bars denote SE of the mean.

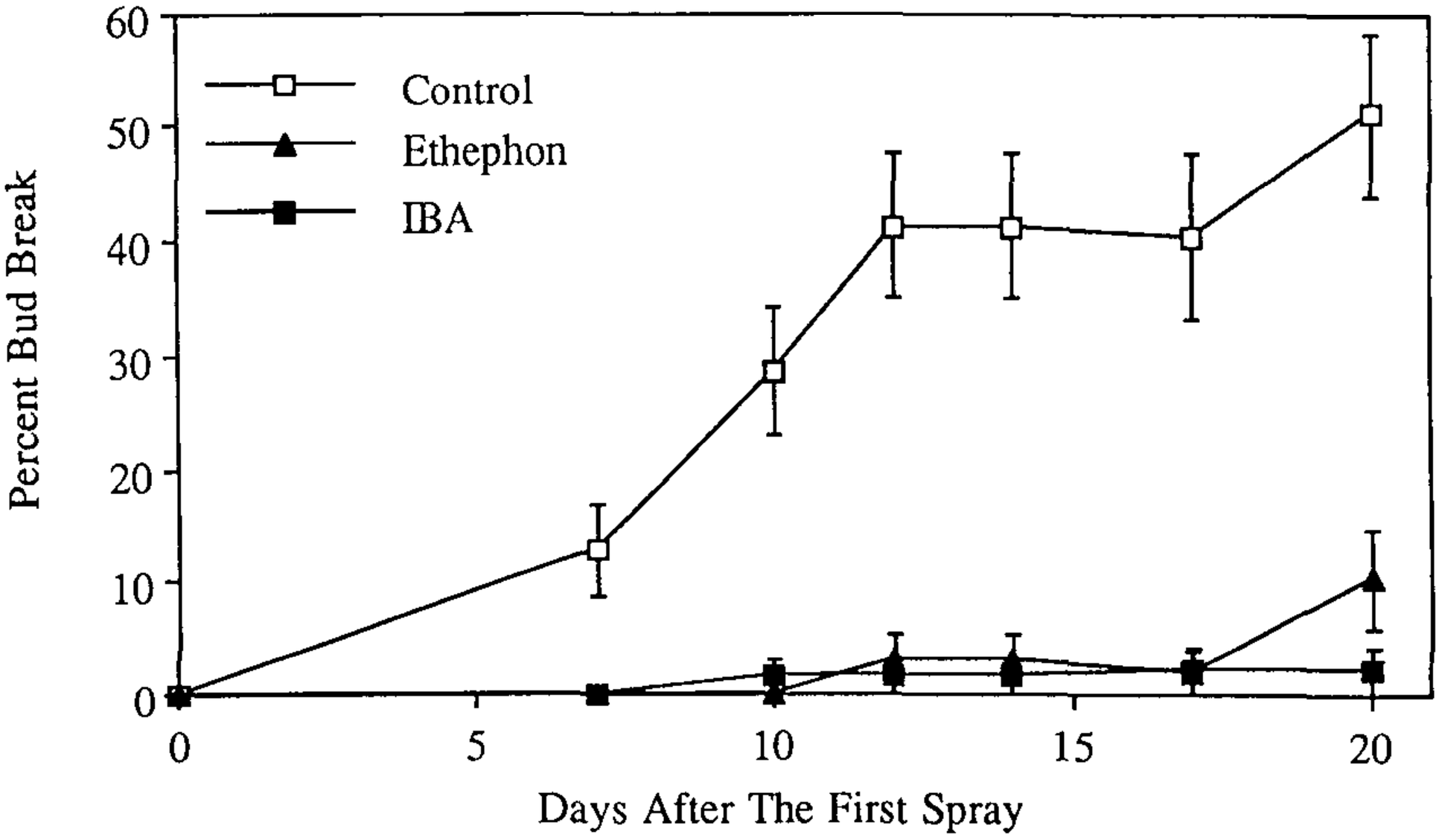

Figure 4. Bud break of 'Royalty' rose cuttings following IBA and ethephon application. IBA at 600 mg·liter^{-1} was applied through 10 sec dip before rooting, and ethephon at 500 mg·liter^{-1} was sprayed on leaves of cuttings daily at 10:00 a.m. at the first 10 days. Data are means of four replications of 15 to 16 cuttings. Bars denote SE of the mean.

DISCUSSION

IBA-induced root formation in rose cuttings was accompanied with increased ethylene levels. Ethylene levels were always inversely correlated with bud break (Fig. 1, 2, 4). STS, an ethylene action inhibitor, stimulated bud break of cuttings in absence of IBA, and increased the early bud break of IBA-treated cuttings (Fig. 5).

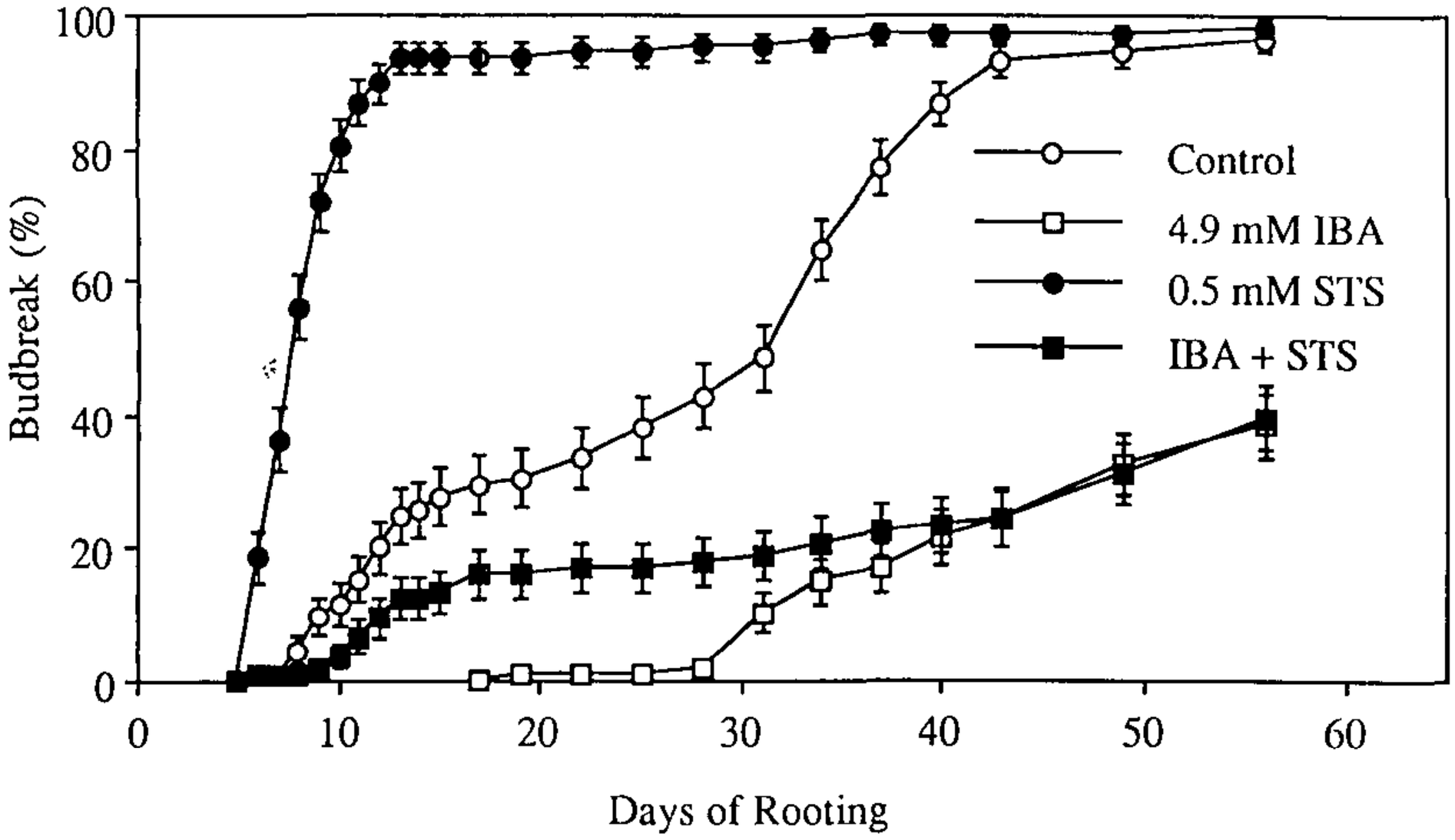

Figure 5. Bud break in 'Royalty' rose cuttings after STS and IBA treatments (Experiment A). Bars indicate SE of the mean (N = 120).

In prior work, ethylene applied to pea nodal sections and decapitated stem cuttings effectively retarded axillary bud development. Buds lost their ability of further development when ethylene treatment lasted more than 3 days (Burg and Burg, 1986). IBA treatment dramatically increased ethylene production of rose cuttings over the first week (Fig. 3). Significant differences in ethylene levels between the control and IBA-treated cuttings was still apparent even after 20 days. In pea plants, stem ethylene concentration decreased after decapitation which stimulated the outgrowth of lateral buds. Ethephon suppressed axillary bud development when it was applied to nodes, axillary buds or the cuts of decapitated plants (Yeang and Hillman, 1982).

Recent work conducted in two laboratories provided further support for our hypothesis. Wiesmen et al. (Wiesman et al., 1989; Wiesman et al, 1989) showed that IBA applied at the cutting base was more likely to be transported to the upper part of the cutting and rapidly metabolized into IBA conjugates, which were even superior to free IBA in serving as the auxin source during the later stages of rooting. Moreover, Riov and Yang (1989) observed that IBA-treated mung bean cuttings had higher levels of ethylene precursors and ethylene in the upper part of the cutting during rooting. These studies, together with our present results, suggest that applied auxin was transported to the upper part of the cutting, increased ethylene production, and as a result, inhibited bud break of cuttings. Auxin-induced ethylene synthesis is primarily responsible for the bud break inhibition of auxin-treated cuttings.

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FRASER HANCOCK: Is the leaf drop that occurs with *Ilex* during cutting propagation related to ethylene?

NINA BASSUK: It could very well be that your increasing the ethylene level which may be increasing the senescence of the leaves.

STEVE MCCULLOCH: Did you notice any carrier effect differences.

NINA BASSUK: No.