not only suggest that exogenous iP induces the adventitious formation of TP(+) and abnormal shoots, but also that the cytokinin affects shoot morphology long after transfer to hormone-free medium. Above 30 mM iP, no increase in leaf death or leaf explants with TP(+) shoots was observed.

Because exogenous iP influences the growth of TP tumors and induces adventitious TP(+) shoots to develop, the uptake and metabolism of exogenous iP was studied by using radioactively-labeled iP ([3H]iP) as a tracer. The [3H]iP (specific activity 6.6 × 104 dpm $pmol^{-1}$) was combined with cold iP to provide a final concentration of 10 mM iP in liquid WP medium. Excised TP(-) and TP(+) shoot tips were placed separately in the medium, and the percent uptake of [3H]iP was calculated from counted aliquots of the medium over 7 days of culture. After 7 days, cytokinins were extracted and prepared for HPLC. Samples were analyzed for cytokinin and adenine nucleotides by anion exchange HPLC. Fractions corresponding to free base and conjugated cytokinins were collected, and prepared for analysis by reverse-phase HPLC. Radioactive peaks were quantified by an IN/US on-line liquid scintillation counter. Our results showed that the percent uptake of [3H]iP increased greatly between 1 and 3 days of culture, with 85% to 90% uptake by the 7^{th} day of culture. Analysis after extraction did not show significant differences in the uptake of iP between TP(-) and TP(+) shoots. The cytokinin metabolites adenosine 5' monophosphate, a glucoside conjugate of iP (IP9G), and iP were identified in both TP(+) and TP(-) shoots. However, only iP levels were significantly different between the two shoot types. In both shoots, greater than 50% of the iP was conjugated to IP9G, resulting in inactivation. Overall, TP(+) shoots appear to metabolize iP faster than TP(-) shoots by 7 days of culture.

The differences in the growth of TP(-) of TP(+) tissues in response to exogenous iP and differences in the metabolism of iP between TP(-) of TP(+) shoots suggest that changes in endogenous cytokinins could be producing the tumorous morphology in *R.* 'Montego'. Currently, we are measuring endogenous cytokinins in TP(-) and TP(+) tissues using an ELISA method.

Long-Term Inhibition of Stem Elongation of *Rhododendron* and *Kalmia* by Triazole Growth Retardants

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That a growth retardant chemical, in combination with a cold- and day-length forcing treatment, could induced *Rhododendron* to flower a year after propagation, was first shown by Stuart (1960). The triazole growth regulators more effectively reduce stem elongation than the chemicals used previously (Davis et al., 1988). Two of these, paclobutrazol and uniconazole, promoted flowering of field-grown *Rhododendron* and *Kalmia* (Gent, 1995a, Ranney et al., 1994; Wilkinson and Richards, 1991), where other chemicals had inconsistent effects. However, paclobutrazol inhibited stem elongation a year after application when applied to *Rhododendron* (Ranney et al., 1994; Wilkinson and Richards, 1991) and *Vitis* (Reynolds and Wardle, 1990), and for 2 years when applied to *Malus* (Williams 1984). This

persistent inhibition of growth by triazole growth retardant could be a problem for woody ornamentals grown by nurseries. A treatment to promote flowering of *Rhododendron* and *Kalmia* could severely inhibit stem elongation for several years, and delay establishment of these plants in the landscape.

Why does the effect of triazole growth retardant persist for several years in woody plants? Inhibition of stem elongation in herbaceous plants, such as *Dendranthema* (syn. *Chrysanthemum*), *Euphorbia pulcherrima* (poinsettia), and annual bedding plants, persisted only for a few weeks after growth retardant was applied. The normal rate of stem elongation in *Dendranthema* was restored as the concentration of growth regulator chemical in plant tissue was diluted by plant growth and increase in biomass (Dicks and Edwards, 1973). Growth of herbaceous plants is rapid and thus the effect of growth retardant would be rapidly diluted. Because woody plants grow more slowly, the effect of growth retardant could persist for a much longer time.

Inhibition of stem elongation is proportional to dose when growth retardant is applied at a low dose, but it is almost completely inhibited at a high dose, and additional growth retardant has little effect. More generally, stem elongation is inversely related to concentration of growth retardant, decreasing to a limiting value as the concentration increases. *Rhododendron* and *Kalmia* responded to triazole growth regulators at doses on the order of 1 mg per plant (Gent, 1995a). Consequently, doses of 10 to 100 mg per plant would completely inhibit stem elongation. When applied at these rates, it would take several doublings in size, e.g., several years, before the chemicals were diluted into the range where stem elongation was only moderately inhibited.

Model. The following equation related the length of stem elongation to the dose of growth retardant applied and the year after application:

$$\text{Growth (dose , year)} = \text{initial growth} + \frac{\text{Growth(dose = 0 , year)}}{1 + K_c \cdot \text{dose} \cdot \exp(-K_t \cdot \text{year})}$$

In the year of application, year = 0, some initial growth was not affected by growth regulator. In the years following application, the initial growth was not significantly different than zero.

Growth (dose=0 , year) was the maximum stem elongation in each year, that of untreated plants. A dose-response coefficient was K_c, in units of mg^{-1} plant, specific to the growth retardant chemical, the plant species and cultivar, and the size of the plant. K_t was a time constant, in units of $year^{-1}$, for the exponential decrease in effect of growth retardant. The time constant should only depend on growth rate. Figure 1 shows the predictions for stem elongation, as a percentage of growth of untreated plants, for each of several years after application of a single dose of growth retardant. The prediction used coefficients of $K_C = 2\ mg^{-1}$ plant (e.g., 0.5 mg inhibits half of stem elongation in the year of application) and $K_t = 2\ year^{-1}$ (e.g., the dose response decreases seven fold per year, and a dose of 3.5 mg is required to inhibit half of stem elongation in the year after application).

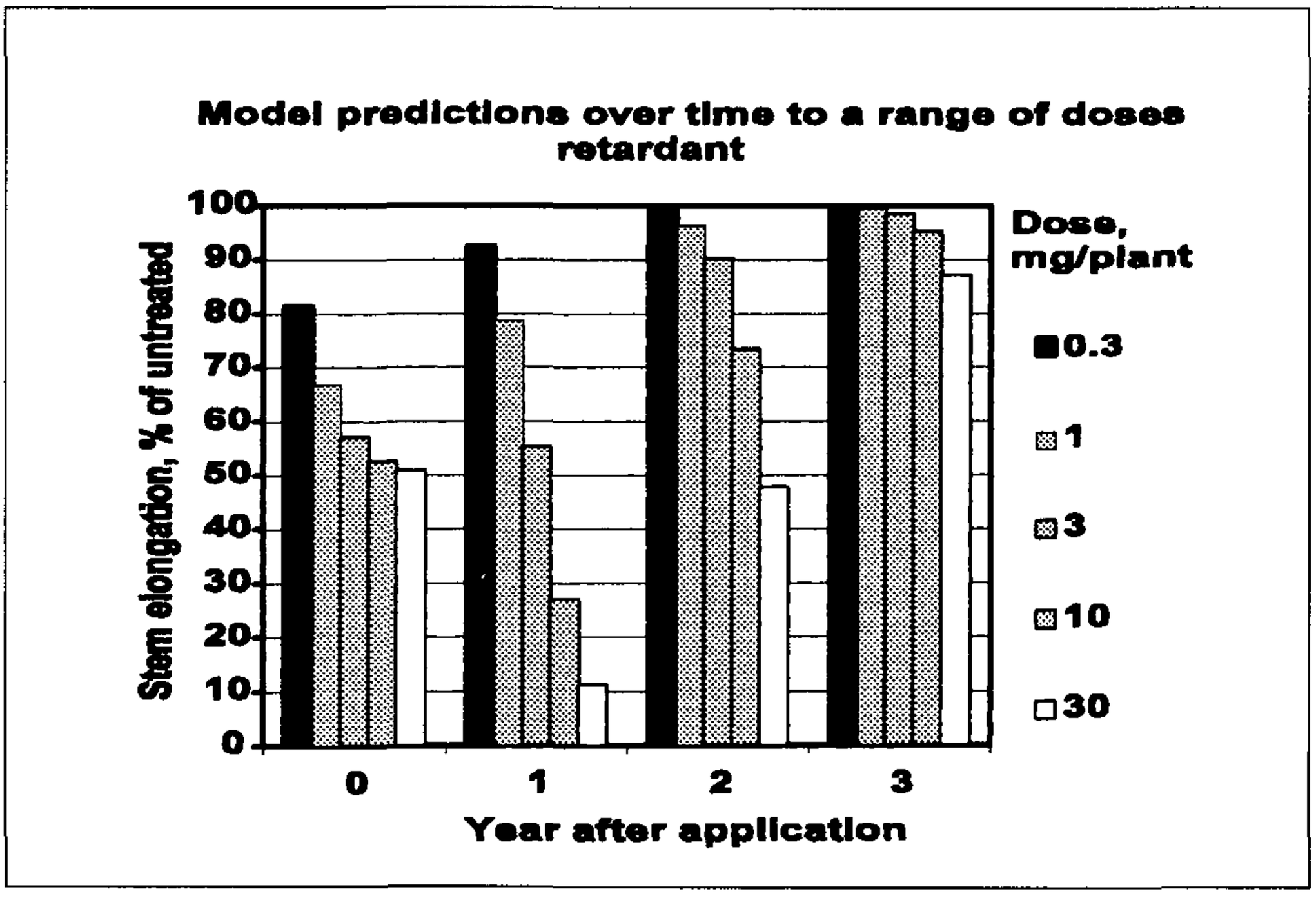

Figure 1. Model predictions over time to a range of doses retardent.

MATERIALS AND METHODS

Plant Material and Growth Conditions. The large-leaf *R. catawbiense* Michx. cultivars 'Boursault' and 'Roseum Elegans', and the *K. latifolia* L. cultivars 'Carousel' and 'Yankee Doodle' were propagated and potted in 8-liter pots at Prides Corner Farm, Lebanon, Connecticut, a commercial nursery. Plants were treated and grown at Lockwood Farm, Hamden Connecticut, the experimental farm of the Connecticut Agricultural Experiment Station. The plants were spaced two-pot diameters apart in full sun, and watered at regular intervals during the growing season, protected over the winter in hoop houses that were covered with white polyethylene film, and transplanted into a field in spring of the year following application of growth retardant. The field was plowed, and 10 : 4 : 8 ($N : P_2O_5 : K_2O$, by volume) fertilizer and powdered sulfur were incorporated to give high fertility and adjust the pH to 5.0. Plants were set in rows with 0.6 m between plants in the row and 1 m between rows. The soil surface was covered with a layer of wood chips. Insecticides, fungicides, and herbicides were applied according to normal production practices.

Application of Growth Regulators. The growth regulators used were paclobutrazol, (2RS,3RS-1-[4-chlorophenyl]-4,4-dimethyl-2-[1,2,4-triazol-l-yl]-pentan-3-ol in the BONZI formulation (Uniroyal Chemical Co., Naugatuck, CT), and uniconazole, (E-1-[4-chlorophenyl]-4,4-dimethyl-2-[1,2,4-triazol-1-yl]-1-penten-3-ol in the SUMAGIC formulation (Valent Chemical Co., Walnut Creek, CA). The plants were treated in the 2nd year after propagation, except the *Kalmia* 'Carousel' treated in April 1992 were in the 3rd year. Each plant received just one dose of growth retardant. In each year, a batch of solution was applied in its entirety to a group of plants. The solution was applied to leaves and stems as a timed, directed spray, with repeated applications, to equalize the volume applied to each plant and

to reduce runoff to a minimum. Six or more plants of each cultivar were not sprayed to serve as controls.

Measurements. In April, three to five stems on each plant were marked with white paint just below the terminal bud. In October, the length of the annual growth was measured for three stems of each plant. Typically, the growth was measured for the longest leader on each of three different branches resulting from pruning in the 1st year of propagation. If there were only two branches, a side shoot was measured. This procedure was repeated in each year following application of growth regulator.

RESULTS

Application in 1991. After a spray application of paclobutrazol in August 1991, stem elongation of *R.* 'Roseum Elegans' in 1991 was inhibited to a similar extent by doses of 5 and 20 mg. In 1992, stem growth was inhibited more by a dose of 20 than 5 mg. In 1993, there was still a significant response to 20 mg paclobutrazol applied in 1991.

In the years following application, stem elongation of *K.* 'Yankee Doodle' was more severely inhibited than that of *Rhododendron*. Stem elongation was inhibited in 1994, 3 years after application of a 20 mg dose of paclobutrazol. This was the only combination of treatment and cultivar that also resulted in smaller leaves than the controls. This illustrated that triazole growth retardant could inhibit stem elongation for at least 3 years after a spray application.

Application in 1992. When growth retardant was applied on three dates in 1992, the initial growth, that growth of stems not inhibited by growth retardant, depended on the date of application. These results have been reported previously (Gent, 1995a). Across application dates, a single coefficient was sufficient to predict the dose response of that part of stem elongation sensitive to growth retardant. Half the maximum inhibition of stem elongation in 1992 was achieved with a dose per plant of less than 0.7 mg paclobutrazol or 0.04 mg uniconazole, except for *K.* 'Carousel'. For all cultivars, and for both chemicals, there was a significant response of stem elongation in 1993 to application of growth regulator in 1992. Because all stem growth was sensitive to growth retardant in the year following application, the inhibition as a percent of total stem elongation of untreated plants was greater than in the year of application. In 1993, stem elongation was inhibited to half that of untreated plants by the residual effect of less than 5.0 mg paclobutrazol and 0.4 mg uniconazole, with the exception of *K.* 'Carousel'. The *K.* 'Carousel' were 3 years after propagation when treated in April 1992, and these larger plants had the lowest dose response. In contrast, *K.* 'Yankee Doodle' was the cultivar most affected by both chemicals, and it grew very little in the year after application, even at low doses.

When the year after application was included in regression to determine the time constant, K_t, for the decrease in the dose response, the time constant for an exponential decrease was 1.3 to 2.7 $year^{-1}$. This corresponded to an apparent decrease in the dose response coefficient, K_C, of 4 to 15 fold per year. In general, the effect of growth retardant persisted for a shorter time in *Rhododendron* than in *Kalmia*. The K_c for uniconazole was 4 to 20 times that for paclobutrazol, but the decrease over time in the dose response was similar for the two chemicals.

Application in 1995. The inverse response of stem elongation to concentration of growth retardant was clearly seen when six doses over a 40- or 50-fold range were applied in 1995. A 0.12 mg dose of uniconazole gave half the maximum inhibition of stem elongation of *R.* 'Boursault' in the year of application. However, only about 5 cm of the total of 15 cm of stem elongation in 1995 was sensitive to application of growth retardant. An inverse response to the dose of growth retardant was also seen in the year following application, but 3.0 mg of uniconazole was required to reduce stem elongation to half that of the control.

In 1995, *K.* 'Carousel' was more responsive to paclobutrazol and uniconazole than was *R.* 'Boursault'. A 0.07 mg dose of uniconazole gave half the maximum inhibition of stem elongation of *K.* 'Carousel', and all but 4 cm of the total of 19 cm of stem elongation in 1995 was sensitive to growth retardant. In 1996, about 0.6 mg uniconazole was required to inhibit half of the stem elongation relative to untreated plants. For both *Rhododendron* and *Kalmia*, the response to a given dose of paclobutrazol was much less than for uniconazole. The effect of paclobutrazol on *R.* 'Boursault' was not significant in the year following application.

DISCUSSION

On average, the apparent response to a particular dose of growth retardant decreased by a factor of seven in each year following application, equivalent to $\mathbf{K_t} = 2\ year^{-1}$. Using this coefficient, the model predicted that a dose inhibiting half of stem elongation in the year of application would only inhibit 12% of the stem elongation in the following year. Practically speaking, the effect of growth retardant would persist for less than a year, if it was applied at this dose. However, a dose 10-fold greater than that required to reduce stem elongation to half that of controls would inhibit stem elongation to less than half that of untreated plants in the following year. This persistent inhibition of stem elongation was nearly always seen after application of a high dose of paclobutrazol or uniconazole. The model predicted a 55-fold decrease in the dose response coefficient by 2 years after application. Spray application of growth retardant had few significant effects in the 2nd year after application, except when high doses were applied to the most sensitive cultivars.

Even for untreated plants, stem elongation was less in the year after application, when plants grew in the field, than in the year of application, when they grew in pots. In part, this difference in growth was due to greater water stress in field-grown than potted plants. This stress would slow the growth in biomass and dilution of growth retardant, and prolong the inhibition of stem elongation. However, stress also decreased the maximum stem elongation and the response to low doses of growth retardant. Thus, the growth conditions in the field did not necessarily emphasize the persistence of effects of triazole growth retardants. In part, growth differed between the year of application and the following years because flowering affected vegetative growth. Vegetative shoots often failed to initiate at stem apices that flowered. All plants were strictly vegetative when growth retardants were applied, but in the following years, many stems terminated in a flower raceme. Often, there was no new vegetative growth on one or two of the three stems selected for measurement. Thus, the average stem elongation was reduced by stems that did not grow at all.

The responses to paclobutrazol and uniconazole were qualitatively similar, but uniconazole was effective at lower doses than paclobutrazol. Based on the regression analysis of the *Rhododendron* treated in 1992 and 1995, the efficacy of uniconazole

was 10 to 20 fold that of paclobutrazol. *Kalmia* differed less in response to the two chemicals. For *Kalmia*, the efficacy of uniconazole was 4 to 8 fold that of paclobutrazol.

Whereas stem elongation showed an inverse relation to dose of growth retardant, the number of flowers per plant generally showed a linear response. After application in April or June 1992, the response of stem elongation to paclobutrazol or uniconazole was non-linear (inverse) in 8 of 16 comparisons, while the number of flowers per plant showed a nonlinear response in only 3 of 16 comparisons (Gent, 1995a). Thus, nearly complete inhibition of stem elongation was achieved more easily than complete expression of flowering. After application in 1995, the number of flowers was not significantly increased for *R.* 'Boursault', and flowering of *K.* 'Carousel' was increased only with high doses of paclobutrazol and uniconazole (Gent, 1995b). In part, plants did not respond in 1995 because untreated plants flowered, which was not the case in 1991 and 1992. In 1992, some flowering was induced by a dose of growth retardant that inhibited less than or equal to half the stem elongation of untreated plants. This dose would have little effect of stem elongation in the following year. Thus, there is a dose of paclobutrazol or uniconazole that can induce flowering when untreated plants would normally not flower, but that will not severely inhibit stem elongation in the following year.

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