

# Comparison of Thidiazuron and Two Nitroguanidines to Kinetin on Potato Microtuberization *in vitro* under Short and Long Days

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## ABSTRACT

Thidiazuron and many nitroguanidines (for example, AC239,604 and AC243,654) act as cytokinins similar to kinetin in many bioassays. Potato tuber initiation effects by thidiazuron, and AC239,604 and AC243,654 have not been investigated. These compounds were compared with kinetin for tuber initiation on nodal segments *in vitro* under short and long photoperiods. Potato plantlets from "Atlantic" tubers were grown *in vitro* under 16-h days on Murashige-Skoog (MS) medium with 2.5% sucrose. After 2 months, nodal segments were removed and cultured on MS medium containing 6% sucrose and a test compound. Half the nodal segments remained under 16-h days (long photoperiod) and half under 8-h days (short photoperiod). Kinetin was added to the MS medium at 2 mg/L. The test treatments were 0.1 mg thidiazuron/L, 0.01 mg AC239,604/L, and 1.0 mg AC243,654/L. Stolon number and length, root length, stem length, time to microtuber initiation, and microtuber size and number were recorded at 5-day intervals up to 80 days after culture. Thidi-

azuron behaved much like kinetin in promoting the percent of nodal segments producing stolons and inhibiting the growth of stems and roots under both long and short photoperiods. Thidiazuron and kinetin delayed the onset of tuber initiation and lessened tuber production compared with untreated nodal segments under long days. Under short days, thidiazuron and kinetin increased the rate of stolon elongation, hastened tuber initiation, and increased tuber production. The two nitroguanidines were not as active on stolons, roots, and stems as kinetin. But, under long days, AC243,654 and, to a lesser extent, AC239,604 increased microtuber production without changing the time to tuber initiation. Under short days, the nitroguanidines, as with kinetin, also hastened the onset of tuber initiation but did not increase the number of microtubers produced. Thidiazuron acted like kinetin, promoting tubers under short days, but the nitroguanidines acted differently, promoting tuber production primarily under long days.

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## INTRODUCTION

Purine-type cytokinins have been known to play a role in tuber initiation in potatoes since the 1960s

(Palmer and Smith 1969). Kinetin is a synthetic cytokinin and promotes tuber formation on potato parts such as leafless nodal stem segments in vitro (Forsline and Langille 1976). Because of its established effect on potato tuberization in vitro, kinetin was used as the standard in comparing thidiazuron and two nitroguanidines.

Thidiazuron is a phenylurea derivative exhibiting cytokinin-like activity in tissue culture systems (Mok and others 1987). In structure-activity studies, thidiazuron was the most active cytokinin of the phenylurea derivatives in cytokinin-dependent callus cultures (Mok and others 1982). The structure of phenylureas such as thidiazuron, is markedly different from that of purine-type cytokinins such as kinetin (Fellman and others 1987). Although thidiazuron behaves like the purine-type cytokinins in most bioassays, its behavior is different in the micropropagation of woody plants (Fellman and others 1987) and in habituating *Phaseolus lunatus* callus cultures (Capelle and others 1983). Suggested modes of action of thidiazuron include conversion of nucleotides to nucleosides (Capelle and others 1983), promotion of purine cytokinin synthesis (Thomas and Katterman 1986), and inhibition of cytokinin oxidase, which deactivates natural cytokinins (Hare and Van Staden 1994).

Benzyl and phenyl nitroguanidines make up a new family of compounds with cytokinin-like activity in many in vitro and in vivo systems (Speltz and others 1990). AC239,604 (1-(3,5-dichlorophenyl)-3-nitroguanidine) increased tobacco leaf size (Pavlista and Templeton 1987), inhibited vegetable leaf senescence (Pavlista 1990), and induced apple bud break (Wang and Faust 1989). AC243,654 (1-(*m*-methoxybenzyl)-3-nitroguanidine) increased yields of several crops (Speltz and others 1990), most notably potato (Pavlista 1994). In greenhouse studies, AC243,654 hastened potato tuberization in vivo (Pavlista 1993). These compounds represent a new class of plant growth regulators with cytokinin activity and are structurally dissimilar from either the phenylureas or purinelike cytokinins.

The main objective of this study was to determine whether the cytokinin-active compounds thidiazuron and the two nitroguanidines, AC239,604 and AC243,654, would stimulate earlier and greater potato tuberization in vitro as with kinetin. Do these compounds also stimulate stolon production as does kinetin and inhibit root and stem growth as well? An additional objective was to determine whether tuberization and the other vegetative growth parameters are affected by the cytokinin-active compounds under short and long photoperiods in the same way.

## MATERIALS AND METHODS

### Plantlet Preparation

Potato plantlets (*Solanum tuberosum* L. "Atlantic") were cultured and micropropagated in test tubes (25 × 150 mm) containing 15 mL of gelled (0.8% Difco-Bacto agar Murashige and Skoog (MS) basal medium at pH 5.8 (Murashige and Skoog 1962) supplemented with 2.5% sucrose. These were placed under a long-day photoperiod of 16 h under a light intensity of 32  $\mu\text{mol s}^{-1} \text{m}^{-2}$  at the top of the tubes using cool-white fluorescent lights and at temperatures of 25 ± 1.0°C day and 22 ± 1°C night.

### Experimental Conditions

After 2 months, single nodal segments from plantlets were placed in Magenta GA7 boxes (Magenta Corp., Chicago, IL) containing 65 mL of MS medium containing 6% sucrose (pH 5.8), untreated control, or supplemented with either thidiazuron (TDZ) or a nitroguanidine, AC239,604 and AC243,654, or, as an active check, kinetin (KIN). Four nodal segments were cultured in each Magenta box and each treatment was replicated 10-fold, that is, 10 boxes (40 nodal segments total) per treatment. The short-day photoperiod was 8 h under 34  $\mu\text{mol s}^{-1} \text{m}^{-2}$  light intensity (top of boxes) at 25 ± 1.5°C day and at 21.5 ± 0.5°C night. The long-day photoperiod was 16 hours under 31  $\mu\text{mol s}^{-1} \text{m}^{-2}$  light intensity at 27.5 ± 1.5°C day and at 23 ± 0.5°C night. Light was provided by cool-white fluorescent lights. The experimental design was split-plot with photoperiod as the main plot. The compound treatments were replicated 10-fold and completely randomized under each photoperiod. The number of nodal segments producing stolons and microtubers was visually recorded every 5 days up to 80 d in culture. Root, stem, and stolon lengths, and microtuber size were destructively measured after 80 d in culture. The number of days passed before tuber formation was observed and recorded for all stolons. Data were analyzed using SAS-ANOVA with least significant difference (LSD).

### Plant Growth Regulators

Optimum concentrations for TDZ and the two nitroguanidines were determined in preliminary experiments on in vitro tuberization of "Atlantic" potato. The active check was 6-furfurylaminopurine (KIN) at 2 mg/L; the test treatments were *N*-phenyl-*N*-1,2,3-thiadiazol-5-ylurea (TDZ; AgrEvo, Wilmington, DE) at 0.1 mg/L, 1-(3,5-dichlorophenyl)-3-nitroguanidine (AC 239,604; American Cyanamid

**Table 1.** Influence of thidiazuron, AC239,604, AC243,654, and kinetin on growth from nodal segments of potato cv. "Atlantic" plantlets grown under long and short days in vitro for 80 days

Treatments	Root length mean (mm)	Stem length mean (mm)	Microtuber diameter mean (mm)	Microtuber fresh wt. mean (mg)
a) <i>Short days</i> (8-h photoperiod)				
Control	138	118	4.1	86
Kinetin	41	45	4.5	84
Thidiazuron	65	52	4.9	98
AC239,604	118	120	4.4	88
AC243,654	108	133	4.1	89
LSD (0.05)	18	13	NS	NS
b) <i>Long days</i> (16-h photoperiod)				
Control	114	82	7.7	371
Kinetin	86	35	5.1	139
Thidiazuron	66	41	5.4	132
AC239,604	104	74	7.5	244
AC243,654	101	74	7.6	288
LSD (0.05)	26	11	1.2	100
<i>p</i> Values				
Treatments (TR)	**	**	**	*
Photoperiod (PP)	NS	**	**	**
TR × PP	**	**	**	**

\*Significant at  $p < 0.02$ .  
\*\*Significant at  $p < 0.01$ .  
NS, not significant at  $p < 0.10$ .

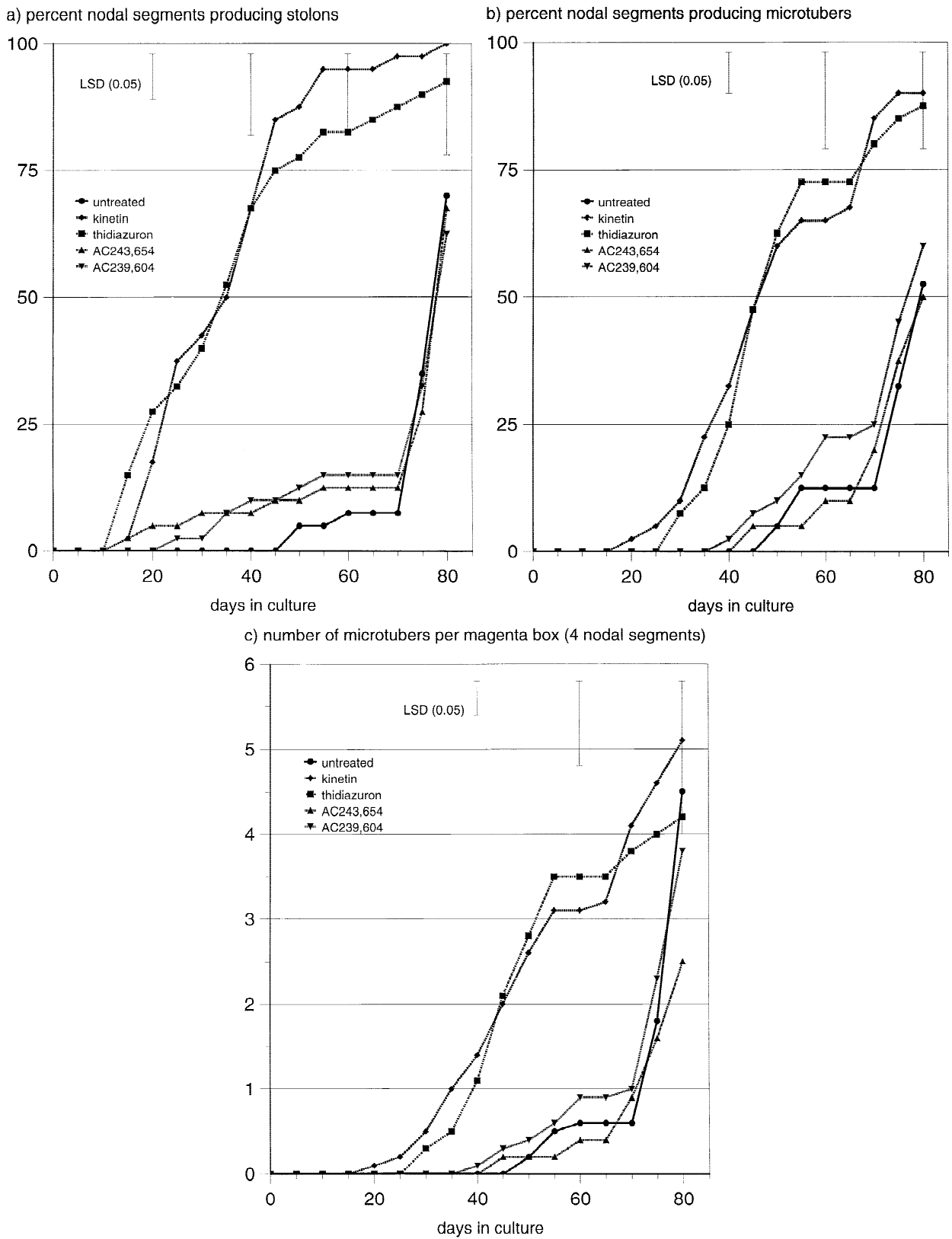
Co., Princeton, NJ) at 0.01 mg/L, and 1-(*m*-methoxybenzyl)-3-nitroguanidine (AC 243,654; American Cyanamid Co., Princeton, NJ) at 1.0 mg/L. KIN, TDZ, AC239,604, and AC243,654 were filter sterilized before adding to autoclaved media.

## RESULTS

### Short Day Photoperiod (8 h)

Root and stem length were significantly less with TDZ and KIN than with untreated controls 80 d after culture (Table 1). The time course of stolon production shows earlier stolon formation and increased percent of nodal segments producing stolons by thidiazuron (TDZ) similar to that by KIN (Figure 1a) and significantly greater than that by the nitroguanidines or untreated control. Stolon number per magenta box (four nodal segments) was not significantly affected (Table 2). The time to observe the first initiation of microtubers was significantly advanced by TDZ and KIN by 10 to 30 d compared with

the control and nitroguanidines (Figure 1b,c), and the average time to tuberization was shorter by 20 to 33 d compared with the control and nitroguanidines (Table 2). Although there was no significant difference in final stolon length, the rate of stolon growth (stolon length in mm divided by the number of days to tuberization) was significantly increased by KIN (90%) and TDZ (170%) over controls (Table 2). After 80 d in culture, there was no significant difference on microtuber growth, diameter, and weight because of TDZ or KIN (Table 1). Earlier tuberization might be expected to result in larger tubers because of a longer growing time. TDZ and KIN greatly promoted the formation of microtubers during the 80 d in culture by an increase in the percent of nodal segments producing microtubers (Figure 1b) and total number of microtubers produced (Figure 1c). The increased number of microtubers with no change in mean microtuber weight resulted in an increase in yield from 321 mg tuber fresh weight per magenta box (four nodal segments per box) to 422 and 400 mg/box for TDZ and KIN, respectively.



**Figure 1.** Under short, 8-h, photoperiod, effects of thiazuron, AC239,604, AC243,654, and kinetin on potato nodal segments: (a) percent nodal segments producing stolons, (b) percent nodal segments producing microtubers, and (c) the number of microtubers produced.

**Table 2.** Influence of thidiazuron, AC239,604, AC243,654, and kinetin on stolon growth and tuber initiation from nodal segments of potato cv. "Atlantic" plantlets grown under long and short days in vitro for 80 days

Treatments	Stolon number per box	Stolon length mean (mm)	Time to tuber initiation mean (d)	Rate of stolon elongation mean (mm/d)
a) <i>Short days</i> (8-h photoperiod)				
Control	10	14	65	0.21
Kinetin	11	13	33	0.40
Thidiazuron	10	21	38	0.57
AC239,604	8	10	58	0.19
AC243,654	8	14	66	0.22
LSD (0.05)	NS	6	10	0.14
b) <i>Long days</i> (16-h photoperiod)				
Control	3.3	27	41	0.71
Kinetin	8.5	33	48	0.71
Thidiazuron	7.4	34	53	0.67
AC239,604	3.3	39	45	0.88
AC243,654	1.7	26	39	0.68
LSD (0.05)	2.5	NS	8	NS
<i>p Values</i>				
Treatments (TR)	**	NS	**	NS
Photoperiod (PP)	**	**	**	**
TR × PP	NS	NS	**	*

\*Significant at  $p < 0.02$ .  
\*\*Significant at  $p < 0.01$ .  
NS, Not significant at  $p < 0.10$ .

Under short days, AC243,654 slightly decreased root length and increased stem length measured after 80 d in culture (Table 1). Earlier initiation of stolon formation on nodal segments was attributed to both nitroguanidines, but the percent of nodal segments producing stolons did not substantially increase after the initial production until 70 d in culture (Figure 1a). Stolon number was not significantly affected (Table 2). Unlike KIN and TDZ, the nitroguanidines did not affect the rate of stolon elongation (Table 2). The percent of nodal segments producing microtubers was not changed by the nitroguanidines (Figure 1b) nor was the number of microtubers produced (Figure 1c). The first tuber initiation was observed 5 to 10 d before the control (Figure 1b), but average time to tuberization was unchanged (Table 2), unlike TDZ and KIN. The diameter and weight of microtubers at 80 d in culture were not affected by the nitroguanidines (Table 1).

#### Long Day Photoperiod (16-h)

TDZ behaved much like KIN. After 80 days in culture, root and stem length were decreased compared

with untreated control (Table 1). The percent of nodal segments producing stolons (Figure 2a) was increased, but the growth rate and final length of stolons were not different from control (Table 2). KIN and TDZ significantly increased the total number of stolons produced per magenta box (four nodal segments) (Table 2). The growth of microtubers, diameter and fresh weight, was delayed by TDZ and KIN (Table 1) possibly because of a 5 to 10 d delay in the onset of microtuber initiation (Figure 2b,c). The percent of nodal segments producing microtubers (Figure 2b) and the total number of microtubers produced in a magenta box containing four nodal segments (Figure 2c) were slightly less for TDZ and KIN treatments than control.

The nitroguanidines, AC239,604 and AC243,654, showed no significant effects compared with untreated controls in root, stem, and stolon elongation (Table 1 and 2). The time course readings indicate that the nitroguanidines advanced the percent of nodes producing stolons (Figure 2a) but did not increase the total number produced (Table 2). AC243,654 did not alter the time to first tuberization

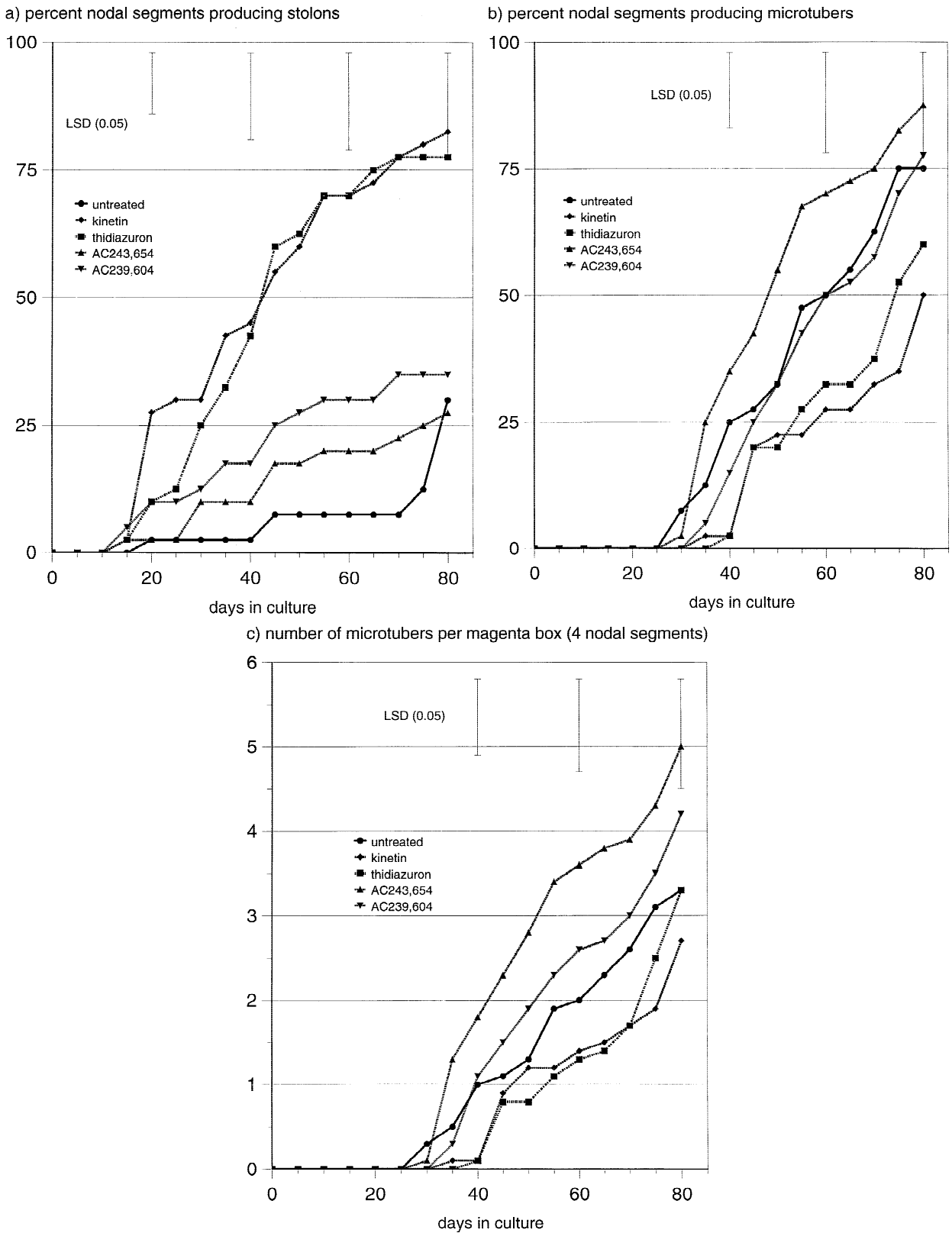


Figure 2. Under long, 16-h, photoperiod, effects of thiazuron, AC239,604, AC243,654, and kinetin on potato nodal segments: (a) percent nodal segments producing stolons, (b) percent nodal segments producing microtubers, and (c) the number of microtubers produced.



(Figure 2b,c), the average time to tuberization (Table 2), nor the diameter and weight of microtubers after 80 d in culture (Table 1). However, AC243,654 ("tuber-promoting" nitroguanidine; Pavlista 1994) significantly increased the percent of nodal segments producing microtubers (Figure 2b) and the number of microtubers produced over the control, KIN (the cytokinin check), and TDZ (Figure 2c). These increases were not observed under short days (Figure 1). AC239,604 ("leaf-promoting" nitroguanidine; Pavlista and Templeton 1987) was not significantly different from control in promoting microtuberization.

### Interactions

Statistical (*F* test) information on the main effects of treatments and photoperiods and their interaction are given in Tables 1 and 2 on early growth of stem nodal segments. Interaction between test compounds (TR) and day length (PP) were significant for all parameters except stolon length. Least significant difference (LSD at  $p = 0.05$ ) bars are shown on Figures 1 and 2. *F* test results for selected times after culture in these Figures showed interactions between test compounds (TR) and day length (PP) were significant ( $p < 0.01$ ) for microtuber production at 40, 60, and 80 days after culture. TR and PP interaction on stolon production was also significant at 20, 40, and 60 days after culture, but not at 80 days.

## DISCUSSION

The promotion of potato tuberization by cytokinins such as kinetin and benzyl adenine, applied to stolons and other organs in vitro is well established (Palmer and Smith 1969). This promotion is greater with organs under short days (8-h light) than under long days (16-h light) (Hussey and Stacey 1984; Pelacho and Mingo-Castel 1991). TDZ and KIN advancement and increase of microtuberization is in agreement with these reports of cytokinin effects. Tuberization under long days was delayed and inhibited by these compounds but, under short days, tuberization was advanced and promoted. Microtuber diameter and weight were reduced under long days but unaffected under short days. KIN has also been reported to inhibit root formation on cultured stolons (Pelacho and Mingo-Castel 1991) and nodal explants (Simko 1993) and stem and leaf development on stem nodal segments (Hussey and Stacey 1984). Agreeing with these findings is the observation that TDZ and KIN inhibited root and shoot growth under both photoperiods.

Although TDZ, a phenylurea, has been tested for cytokinin activity in many systems (Mok and others 1987), its effects on potato are only now beginning to be reported. With stem nodal segments in vitro, TDZ behaves like KIN, a purine derivative. Early experiments with TDZ on field-grown vegetables indicate that this compound will promote yields of potato, as well as other crops (Pavlista unpublished). The two nitroguanidines, AC243,654 and AC239,604, acted differently compared with TDZ and KIN. They increased the number of tubers formed under long days but had little effect under short days. Microtuber size was affected little, even under long days. The nitroguanidines had less of an effect on root or stem growth and stolon formation under either photoperiod compared with TDZ and KIN.

The nitroguanidines, which are structurally dissimilar from purines and phenylureas, have been evaluated as cytokinins in several systems (Speltz and others 1990). AC243,654 promoted tuberization in vivo (Pavlista 1993) and increased field yields (Pavlista 1994). As reported here, AC243,654, has activity similar to kinetin but under different conditions and to a different extent. More tests are needed to relate nitroguanidines to other compounds showing cytokinin activity. There may be several explanations for the differing activities between KIN and TDZ versus the nitroguanidines under the two photoperiods. KIN applied to tissue may be deactivated by light, possibly by promoting gibberellins resulting in greater activity under short days (Pelacho and Mingo-Castel 1991; Simko 1993). No deactivation of TDZ or nitroguanidines has been reported. KIN's activity in tuberization has been reported to be associated with ethylene biosynthesis (Hussey and Stacey 1984; Simko, 1993). TDZ also stimulates the biosynthesis of ethylene (Suttle 1986; Yip and Yang 1986). Ethylene release by nitroguanidines is not yet reported. Another possibility is differences in effects on enzyme activities such as invertase. It is still not known whether nitroguanidines act directly as cytokinins or stimulate natural cytokinin activity, synthesis, or transport.

Since the 1950s (Chapman 1958; Gregory 1956), potatoes have generally been considered short-day plants for tuberization (Forsline and Langille 1976; Pelacho and Mingo-Castel 1991; Slimmon and others 1989). However, there are several reports that long-day exposure of stem nodal segments in vitro may induce more tuberization than short days when 6–8% sucrose was present in the medium (Hussey and Stacey 1981; Perl and others 1991). The reason(s) for these contrasting observations is not clear, but a few explanations have been forwarded: (a)

sucrose requirement (Garner and Blake 1989; Perl and others 1991), (b) ethylene production (Hussey and Stacey 1981; 1984; Mingo-Castel and others 1974), (c) radiance (Bodlaender 1963; Wheeler and Tibbitts 1986), and (d) maturity groupings and commercial breeding (Bodlaender 1963; Ewing and Wareing 1977; Seabrook and others 1993). Perl and others (1991) suggested that high sucrose levels are needed to signal tuber induction and that exposure to long days promotes a second "stabilization" phase before tuberization occurs.

## CONCLUSIONS

In conclusion, TDZ affected vegetative growth and tuberization of stem nodal segments *in vitro* much as KIN did under both short (8 h) and long (16 h) photoperiods. The nitroguanidines acted differently from KIN and TDZ. AC243,654 exerted its promotive influence on tuberization under long days. Microtuber multiplication in seed production would be greatly improved by the addition of KIN or TDZ under short days and by AC243,654 under long days.

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