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Hormonal Regulation of Lateral Bud (Tiller) Release in Oats (*Avena sativa* L.)[1]

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ABSTRACT

Stem segments containing a single node and quiescent lateral bud (tiller) were excised from the bases of oat shoots (cv. 'Victory') and used to study the effects of plant hormones on release of lateral buds and development of adventitious root primordia. Kinetin (10^{-5} and 10^{-6} molar) stimulates development of tillers and inhibits development of root primordia, whereas indoleacetic acid (IAA) (10^{-5} and 10^{-6} molar) causes the reverse effects. Abscisic acid strongly inhibits kinetin-induced tiller bud release and elongation and IAA-induced adventitious root development. IAA, in combination with kinetin, also inhibits kinetin-induced bud prophyll (outermost leaf of the axillary bud) elongation. The IAA oxidase cofactor *p*-coumaric acid stimulates lateral bud release; the auxin transport inhibitor 2,3,5-triiodobenzoic acid and the antiauxin α (*p*-chlorophenoxy)-isobutyric acid inhibit IAA-induced adventitious root formation. Gibberellic acid is synergistic with kinetin in the elongation of the bud prophyll. In intact oat plants, tiller release is induced by shoot decapitation, geostimulation, or the emergence of the inflorescence. Results shown support the apical dominance theory, namely, that the cytokinin to auxin ratio plays a decisive role in determining whether tillers are released or adventitious roots develop. They also indicate that abscisic acid and possibly gibberellin may act as modulator hormones in this system.

The role of plant hormones in the regulation of lateral bud development in dicots has been studied by many investigators (1, 12, 13, 19). However, hormonal regulation of lateral bud release in monocots has received little attention, especially in grasses. Lateral bud (tiller) development in grasses is important agriculturally since increased bud release and consequent tiller development can result in increased yield of certain cereal crops (9).

Growth-regulator substances and nutritional factors are thought to control tiller release. Early studies on the control of tiller release by Leopold (11) indicate that grasses are under the same type of apical control as in dicots, namely, that auxin in the apical bud of the main axis inhibits tillering, and removal of this source by decapitation or by application of the auxin transport inhibitor TIBA[2] releases the buds from apical control. Langer *et al.* (10) found that kinetin stimulates lateral bud release in wheat and suggested that kinetin may act by increasing the flow of assimulates within the shoot to the axillary bud site. They also found that grasses have two phases of tiller development control: one which inhibits buds by apical control and another which releases tillers after the emergence of the inflorescence from the last (flag) leaf.

It is well-established that regeneration of roots at the basal ends of cuttings is regulated by auxin (15-17). Skoog and Miller (14) found that, in tobacco callus tissue cultures, a high auxin to cytokinin ratio stimulates root production.

In light of the fact that hormonal regulation for bud release and adventitious root development in grasses is so poorly understood, it was decided to reinvestigate this problem, using excised oat stem segments and intact oat plants as experimental systems.

MATERIALS AND METHODS

Plant Materials. Oats (*Avena sativa* L. cv. 'Victory') were germinated and grown in a soil mixture of loam-peat-Perlite (4:1:1) in a greenhouse programmed for 20 to 23 C day and 17 C night under natural photoperiod and supplementary illumination by incandescent and influorescent lights, providing a photosynthetic photon flux density of 150 to 1100 μE m^{-2} s^{-1} at plant level for 20 h/day. Plants were fertilized once a week with 300 μg/g nitrogen-phosphorus-potassium fertilizer. Plants 35 to 42 days old were selected for all experiments (with intact shoots and isolated stem segments). At this stage, plants were vegetative.

Experiments with Intact Plants. In these experiments, entire flats of oats were either left upright or placed horizontally for geostimulation treatment. Decapitation of shoots was performed by excising the shoots below the p-node (first node below the peduncle, the main axis of the inflorescence) (Fig. 1). The amount of bud release from the p-5 node was analyzed following geostimulation, decapitation, and treatment with kinetin (10^{-5} M), TIBA (10^{-5} M), PCIB (10^{-5} M), α-naphthaleneacetic acid (10^{-5} M), or IAA (10^{-5} M). For these growth regulation treatments, shoots were sprayed every day for 3 days, and per cent tiller release was determined on the 4th day. Tween 20 (0.1%) (v/v) and dimethyl sulfoxide (0.1%) (v/v) were included in the growth regulator solutions to aid penetration of hormones into shoot tissues. These experiments with intact plants were repeated at least three times.

Experiments with Excised Stem Segments. Segments (2-3 cm in length) containing a single node, a quiescent axillary bud (1.5-2 mm in length) which will form a tiller shoot, and portions of the internode on either side of it were excised below the p-5 node (Fig. 1). These segments were washed thoroughly with distilled H_2O to deter microbial contamination and then placed horizontally or upright in Petri dishes (100 × 15 mm) lined with Whatman No. 1 filter paper saturated with 6 ml incubation medium. The control medium was 0.1 M sucrose. Experimental treatments included sucrose plus hormone (10^{-8} to 10^{-5} M). Hormones or inhibitors employed in these experiments included kinetin, IAA, GA_3, ABA, PCIB, TIBA, and PCA. Twenty segments/dish were incubated in the dark at 25 C for 72 h. Upright segments were placed in raised, circular, perforated Plexiglas holders in Petri dishes (60 × 15 mm) lined with Whatman No. 1 filter paper saturated with 2 ml incubation medium. Apical ends of segments were kept up except

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[2] Abbreviations: TIBA, 2,3,5-triiodobenzoic acid; PCA, *p*-coumaric acid; PCIB, α(*p*-chlorophenoxy)-isobutyric acid.

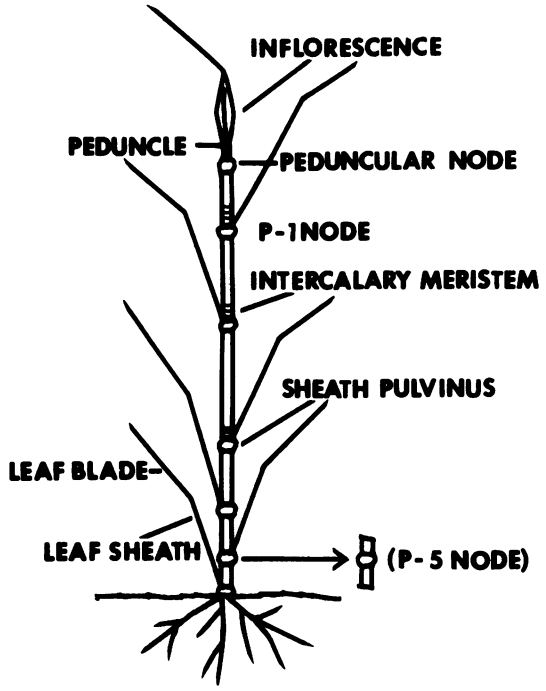

FIG. 1. Diagrammatic representation of an *Avena* plant illustrating the primary parts (flag leaf and inflorescence, peduncle, peduncular node, p-1 node, leaf sheath, sheath pulvinus, and location of the p-5 node).

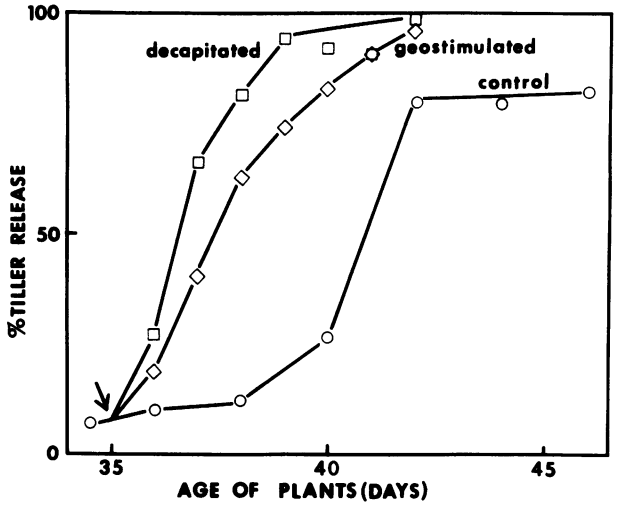

FIG. 2. Tiller release during growth of *Avena* plants, illustrating effects of geostimulation, decapitation, and emergence of the flat leaf and inflorescence. Emergence of the flag leaf and inflorescence occurs in approximately 40- to 41-day-old plants. Arrow indicates time of initiation of geostimulation and decapitation treatments.

for those in auxin treatment. For these, the segments were inverted (apical ends down) since auxin transport in the stem is known to be primarily basipetal (5). All experiments were repeated at least three times.

The release of lateral buds (tiller release) was denoted by the prophyll (outermost leaf of the axillary bud) breaking through the leaf sheath and its tripling in length. Direct prophyll length measurements were also made. The formation of adventitious roots was denoted by the presence of root primordia at the p-5 node, as viewed macroscopically.

Kinetin, IAA, GA_3, ABA, and TIBA were obtained from Sigma. PCIB was a gift from H. Burström, Lund University, Sweden. PCA was a gift from H. Ikuma, University of Michigan, Ann Arbor. 'Victory' oat seed was obtained from the Swedish Seed Association, Svalöf, Sweden.

Table I. *Effect of Partial Periods of Geostimulation on Tiller Release in Intact Oat Shoots*

Per cent tiller release was determined on the 4th day after the initiation of treatments.

Geostimulation Time	Tiller Release[a]
h	%
0	10 ± 1
12	46 ± 5
24	64 ± 5
36	83 ± 3
48	78 ± 2
60	84 ± 5
72	91 ± 5
96	80 ± 8

[a] Mean ± SE.

Table II. *Effects of Growth Regulators Supplied as Foliar Spray to Intact Oat Plants*

Tween 20 and dimethyl sulfoxide were included in the growth regulator solutions. Per cent tiller release was determined on the 4th day after the first treatment. Treatments were applied daily. Growth regulator concentration was 10^{-5} M.

Growth Regulator Supplied	Tiller Release[a]
	%
To whole shoot	
TIBA	54 ± 6
PCIB	44 ± 6
Kinetin	48 ± 9
None	10 ± 1
To decapitated shoots	
IAA	40 ± 0.6
α-Naphthaleneacetic acid	45 ± 4
None	94 ± 5

[a] Mean ± SE.

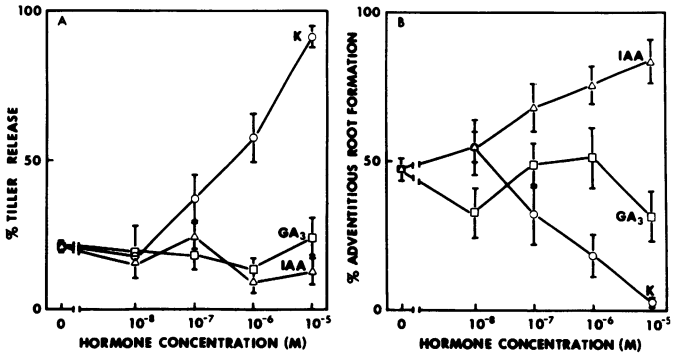

FIG. 3. Effects of kinetin (K), IAA, and GA_3 on tiller release (A) and adventitious root formation (B) in horizontally placed stem segments. Mean ± SE.

RESULTS

Tiller Release in Intact Plants. Figure 2 illustrates the results of experiments on tiller release in intact *Avena* plants following decapitation, geostimulation, and emergence of the flag leaf and inflorescence. Constant geostimulation and decapitation induce 80 and 90% tiller release, respectively, within 4 days after treatment. Emergence of the flag leaf and inflorescence are also associated with an equally rapid tiller release response. Partial

Table III. *Effects of Kinetin, Kinetin Riboside, Zeatin, and Zeatin Riboside on Tiller Release in Isolated Oat Stem Segments Incubated for 72 h at 30 C in Dark*

Hormone Concentration	Tiller Release[a]			
	Kinetin	Kinetin Riboside	Zeatin	Zeatin Riboside
M		%		
10^{-5}	92 ± 3	100 ± 0	50 ± 3	76 ± 5
10^{-6}	58 ± 8	47 ± 4	23 ± 3	42 ± 7
10^{-7}	37 ± 8	23 ± 3	14 ± 3	32 ± 5
10^{-8}	18 ± 3	23 ± 4	14 ± 2	13 ± 1

[a] Mean ± SE.

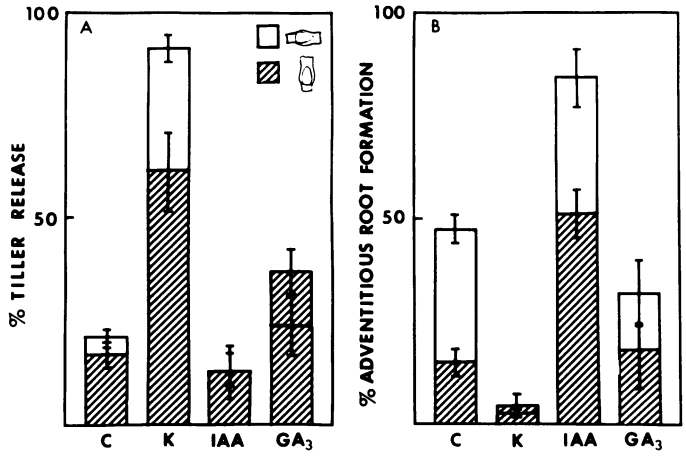

FIG. 4. Effects of kinetin (K), IAA, and GA_3 on tiller release (A) and adventitious root formation (B) in horizontal and upright stem segments as compared to control (C). Hormone concentration, 10^{-5} M. Mean ± SE.

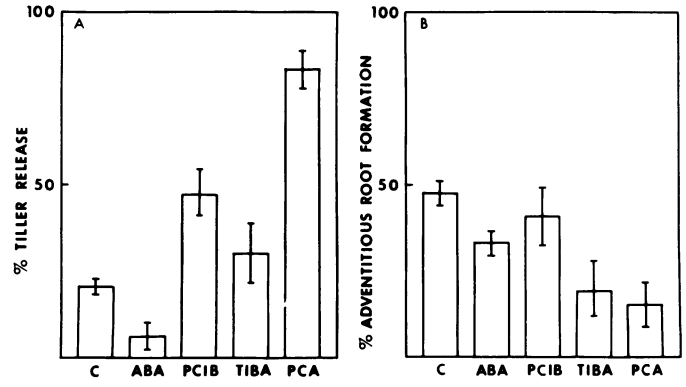

FIG. 5. Effects of ABA, PCIB, TIBA, and PCA on tiller release (A) and adventitious root formation (B) in horizontally placed stem segments as compared to control (C). Hormone concentration, 10^{-5} M. Mean ± SE.

periods of geostimulation for less than 24 h did not induce tiller release over 50% in intact plants (Table I). Applications of foliar sprays of α-naphthalene acetic acid or IAA (applied daily) to decapitated plants each inhibited tiller release 50% compared to decapitated controls 4 days after beginning of treatments. In contrast, tiller release was stimulated 44% above that of intact controls by TIBA, 34% by PCIB, and 38% by kinetin 4 days after the initiation of treatments (Table II). These results indicate that the presence of the apical portion of the shoot inhibits tiller release, presumably by providing auxin to the stem and axillary buds below it. Geostimulation, emergence of the flag leaf and inflorescence, and kinetin may stimulate tiller release, possibly by

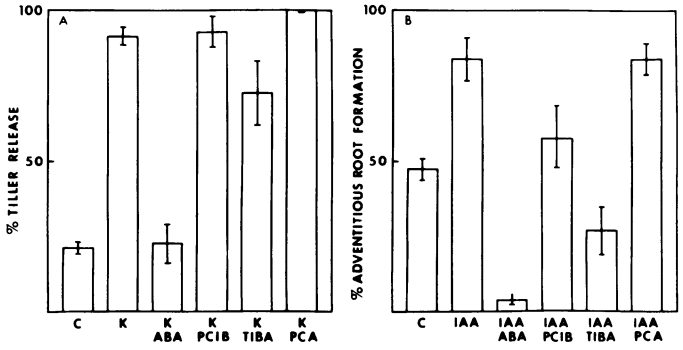

FIG. 6. Effects of ABA, PCIB, TIBA, and PCA on kinetin-induced tiller release and IAA-induced adventitious root formation compared to kinetin (K) and IAA alone. Hormone and inhibitor concentration, 10^{-5} M. Mean ± SE.

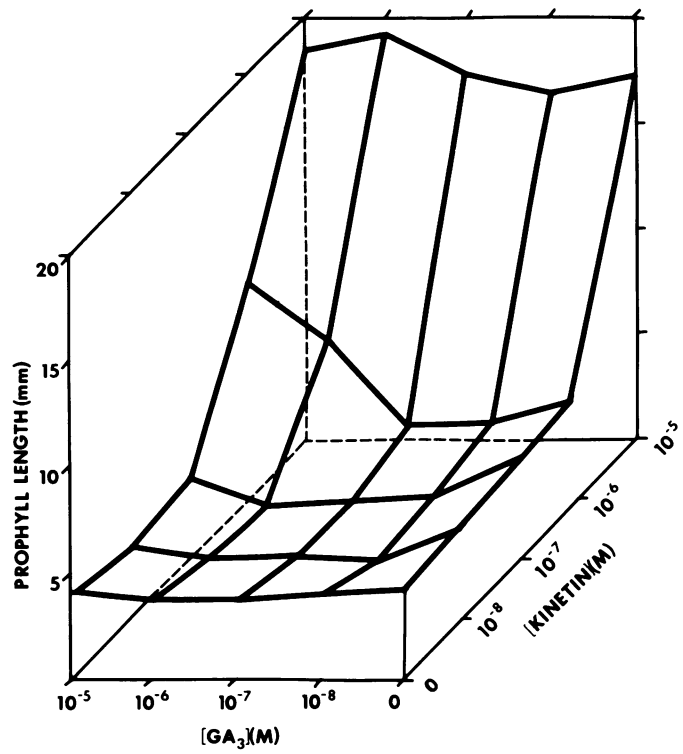

FIG. 7. Interaction of GA_3 with kinetin on prophyll elongation in horizontally placed stem segments.

repressing auxin synthesis and/or basipetal transport.

Effects of Hormones on Bud Release in Excised Stem Segments. Since geostimulation causes tiller release in intact plants, both horizontally placed (geostimulated) and vertically oriented segments were used in experiments with excised stem segments. Figure 3 illustrates results for tiller release and adventitious root development in horizontally placed segments following treatments with kinetin, IAA, and GA_3. Kinetin at 10^{-5} and 10^{-6} M causes a significant increase in tiller release and inhibits adventitious root formation in these stem segments. Zeatin also stimulates tiller release, but to a lesser extent than kinetin (Table III). Kinetin riboside and zeatin riboside stimulate tiller release to a greater extent than the non-riboside forms of the hormones. IAA at 10^{-5} and 10^{-6} M induces adventitious root formation, but it has little effect on tiller release (Fig. 3). GA_3 does not appear to show any significant effects on either process. In upright segments, kinetin, IAA, and GA_3 have similar effects, but the magnitude of the response is less than that obtained in horizontally placed segments

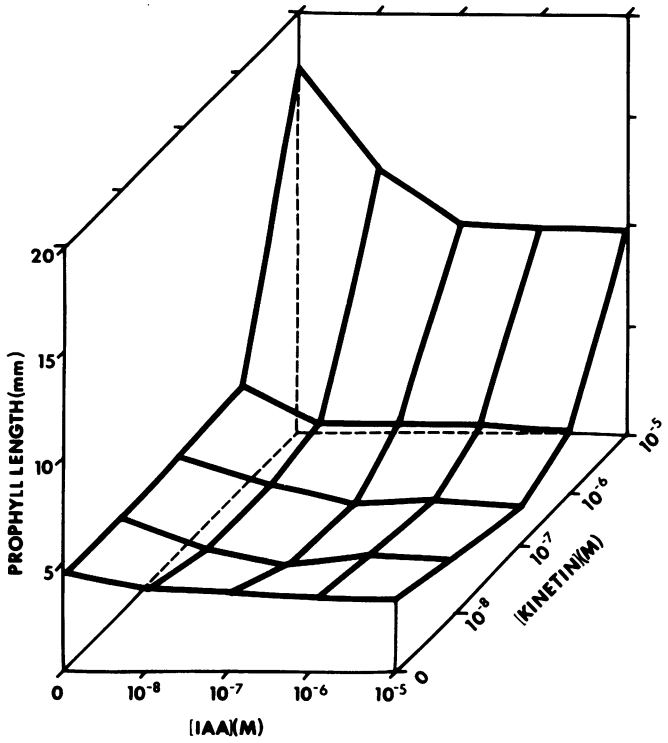

FIG. 8. Interaction of IAA with kinetin on prophyll elongation in horizontally placed stem segments. Scale for IAA concentration is inverted so that no points are hidden on the figure.

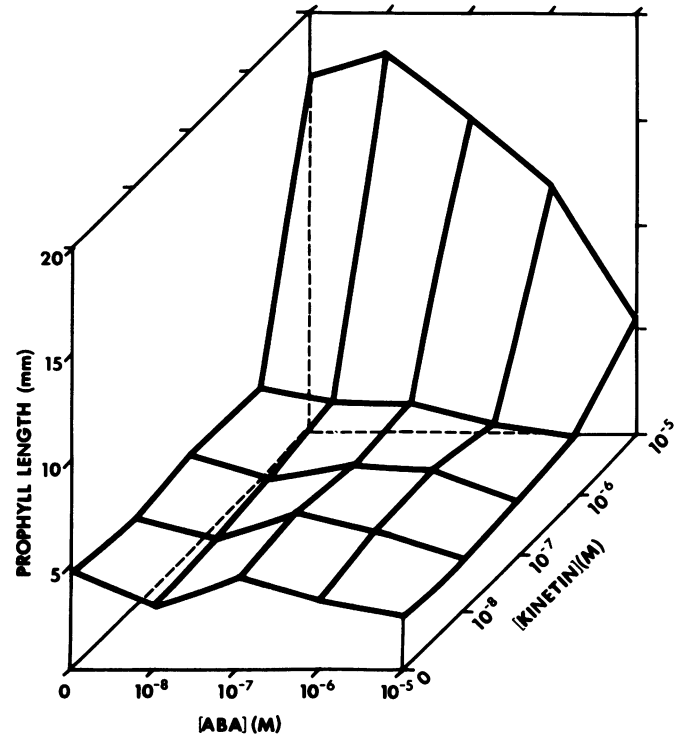

FIG. 9. Interaction of ABA with kinetin on prophyll elongation in horizontally placed stem segments. Scale for ABA concentration is inverted so that no points are hidden on the figure.

(Fig. 4). Figures 5 and 6 illustrate the effect of the auxin inhibitors TIBA, PCIB, and PCA and that of ABA on these processes. TIBA and PCIB did not appreciably affect tiller release. TIBA causes a significant diminution in the number of adventitious root initiated. PCA (an IAA oxidase co-factor) (4) greatly stimulates lateral bud release and inhibits adventitious root formation. ABA strongly inhibits tiller release when used alone (Fig. 5) and in combination with kinetin (Fig. 6). TIBA, PCIB, and ABA significantly reduce the amount of adventitious root formation when used in combination with IAA. These results suggest that cytokinin may be involved in the stimulation and ABA in the suppression of tiller release. The strong promotion by PCA suggests that stimulated auxin oxidation allows for greater tiller release.

Since tiller release is defined by tiller bud prophyll elongation, the interaction of GA_3, IAA, and ABA with kinetin on prophyll growth was also studied. GA_3 exhibits a synergistic effect on prophyll elongation (Fig. 7). However, GA_3 alone does not induce significant tiller release (Figs. 3 and 4). IAA (10^{-5} to 10^{-8} M) inhibits kinetin-stimulated prophyll elongation (Fig. 8). Thus, the cytokinin to auxin ratio may be important in controlling prophyll elongation. ABA also greatly inhibits kinetin-stimulated prophyll elongation but only at higher concentrations (10^{-5} and 10^{-6} M) (Fig. 9).

DISCUSSION

These results, were visualized as the model shown in Figure 10. This model, based on lateral bud release in oat shoots, supports the classical apical dominance theory for control of shoot development that has been promulgated for dicots (19) and for monocots (10). The inhibition by auxin of kinetin-induced tiller release from quiescence and prophyll elongation indicates that the cytokinin to auxin ratio is an important factor in determining whether tillers are released or whether adventitious roots form. Both free and riboside forms of cytokinin are active in inducing tiller release. The riboside forms may be active by themselves or they may be "storage" forms which are not metabolized by the stem tissue as rapidly as are free forms.

Our results indicate that ABA may also be involved in inhibiting tiller bud release and elongation and adventitious root formation. In the presence of ABA and low concentrations of both auxin and of kinetin, little tiller bud release and prophyll elongation occurs. High concentrations of ABA-like substances have been found in Craigella Lateral Suppressor tomato plants (18). Tucker (18) suggested that auxin controls lateral bud development by regulating the concentration of these ABA-like substances in the buds. However, no work has been conducted on the endogenous levels of ABA in *Avena*.

GA_3 also displays a synergism with kinetin on bud prophyll elongation. However, GA_3 alone does not stimulate tiller release from quiescence. Kaufman *et al.* (6) found that the inflorescence and nodes are the major sources of endogenous gibberellins in oats. Gibberellin present in the nodes may contribute to prophyll elongation after tiller release has been initiated.

The stimulation of tiller release by decapitation, geostimulation, and the emergence of the flag leaf and inflorescence most likely involves regulation of the hormonal balance at the lateral bud site. Decapitation could alter the cytokinin to auxin ratio simply by removing the primary source of auxin, namely, the shoot tip (shoot apex and young leaves). The effect of the shoot tip can be replaced in part by exogenous auxin treatment. The fact that auxin treatment could not fully replace the inhibition produced by the shoot tip may be due to IAA oxidation at the cut surface. Also, Field and Jackson (3) have suggested that the shoot tips may synthesize other hormones besides auxin which may be involved in bud suppression. Geostimulated pulvini of oats were found to have 7 times more endogenous free IAA than upright pulvini (7). Also, 2.5 times more free IAA was located in lower halves than in upper halves of the pulvini. Pulvini accumulate IAA asymmetrically and can transport from the upper to lower halves of geostimulated pulvini. In the investigation reported here, all tillers have the ability to release during geostimulation regardless of their position

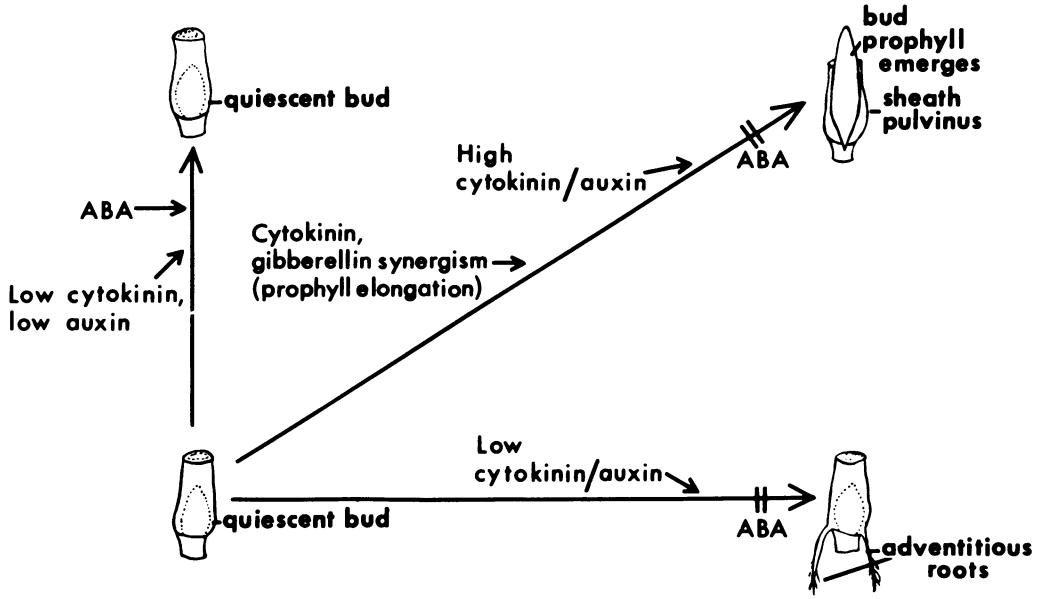

FIG. 10. Proposed model for the roles of ABA, GA_3, and the cytokinin to auxin ratio in tiller release and adventitious root formation.

on the stem. Thus, buds located on the lower side of the stem do not appear to be influenced by lateral transport of auxin to that site. Geostimulation also increases endogenous gibberellins 28 times in lower halves of pulvini relative to upper halves (8). This trend occurs in the internodes as well. This increase in gibberellins in geostimulated oat shoots may contribute to prophyll elongation after tillers are released from quiescence.

In the results presented here, horizontally placed stem segments exhibit a greater degree of response to kinetin than do upright segments. This could be due to differences in transport of hormones in horizontal compared to upright segments. Tiller release during the emergence of the flag leaf and inflorescence may be due to an increase in the amount of cytokinin flow in the xylem from the roots up the stem to inflorescence, as suggested by Beevers and Woolhouse (2). Langer *et al.* (10) also reported that the release of buds at this stage is not associated with auxin since it is not influenced by TIBA treatment.

From work presented here, the cytokinin to auxin ratio appears to play a major role in regulating tiller release and adventitious root formation. The strong inhibitory effect of exogenously applied ABA indicates that ABA may play a role in tiller suppression. The synergism of GA_3 on kinetin-stimulated prophyll elongation suggests that gibberellins may contribute to tiller bud elongation after release from quiescence.

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