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Short Communication

Inhibition of Growth by Ancymidol and Tetcyclacis in the Gibberellin-Deficient *Dwarf*-5 Mutant of *Zea mays* L. and Its Prevention by Exogenous Gibberellin

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Abstract. Leaf sheath length and shoot dry matter of the gibberellin-deficient *dwarf*-5 mutant of *Zea mays* L. were further reduced by micromolar concentrations of two putative gibberellin biosynthesis inhibitors, ancymidol [α-cyclopropyl-α-(p-methoxyphenyl)-5-pyrimidine methyl alcohol] and tetcyclacis [5-(4-chlorophenyl)-3,4,5,9,10-pentaazatetracyclo-5,4,1,$0^{2,6}$,$0^{8,11}$-dodeca-3,9-diene]. Growth retardant action was prevented by the subsequent application of gibberellin (GA_{4+7}). Plants treated with both gibberellin and growth retardants were identical in all outward respects to those treated with gibberellin alone. Although the *dwarf*-5 mutant is blocked in the synthesis of *ent*-kaurene and does not contain detectable quantities of gibberellin, the above results are consistent with the interpretation that biologically active levels of endogenous gibberellin are present in the dwarf which can be decreased by biosynthesis inhibitors.

During studies on the role of GA in carbohydrate metabolism and photosynthate partitioning, it was determined that growth of the *dwarf*-5 mutant of *Zea mays* L. was further inhibited by the addition of micromolar amounts of putative GA biosynthesis inhibitors (ancymidol and tetcyclacis) to a root wash solution (Britz and Saftner, unpublished results). Higher concentrations of the inhibitor CCC were also effective. Although *dwarf*-5 is apparently blocked in

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Abbreviations and nomenclature: GA(s), gibberellin(s); ancymidol, α-cyclopropyl-α-(p-methoxyphenyl)-5-pyrmidine methyl alcohol; CCC, (2-chloroethyl)trimethylammonium chloride; tetcyclacis, 5-(4-chlorophenyl)-3,4,5,9,10-pentaazatetracyclo-5,4,1,$0^{2,6}$,$0^{8,11}$-dodeca-3,9-diene.

the synthesis of *ent*-kaurene (Hedden and Phinney 1979), genetic evidence consistent with the presence of GA in the mutant is provided by crosses between *dwarf*-5 and *dwarf*-1 which are even shorter than the parent strains (Phinney, personal communication). Both genetic dwarfs and chemical growth regulators have been helpful in the study of GA-related physiology, so it is important to assess as completely as possible any limitations to their use. Thus, the following questions arise. Does *dwarf*-5 actually have low but physiologically active levels of GA, and do the growth retardants affect the mutant by blocking GA biosynthesis? It will be difficult to answer these questions directly, because GAs in *dwarf*-5 are either not detectable (Phinney and Spray 1982) or present in only trace amounts (Spray et al. 1984).

A minimal condition that must be met to consider a connection between growth retardant activity and GA biosynthesis is that any inhibition be reversed *completely* by exogenous GA (Lang 1970). There are numerous instances where this condition is not fulfilled. For example, CCC and other putative GA biosynthesis inhibitors (AMO-1618 and Phosfon D) inhibited sterol synthesis in tobacco (Douglas and Paleg 1974). Growth inhibition by these compounds was completely overcome in several cases by exogenously added sterols, whereas it was not by GA_3.

Although ancymidol blocks the oxidation of *ent*-kaurene (Coolbaugh et al. 1978) and was reported not to inhibit sterol biosynthesis (Shive and Sisler 1976), the situation with regard to antagonism by GA is consistent with possible nonspecific effects. Thus, ancymidol (39 μM) inhibition of bean stem growth was fully reversed by GA_7 (2 μM) only in dark-grown seedlings; in light-grown seedlings, the inhibition was reduced from 65% to 49% (Shive and Sisler 1976). In other plants (corn, peas, *Pharbitis*), it is impossible to evaluate the true extent of reversal because the appropriate GA-alone controls were not reported (Leopold 1971, Coolbaugh et al. 1982, Suge 1980). However, in the lettuce hypocotyl test, inhibition of growth by ancymidol (10 μM) was approximately constant (36–42%) over 10^{-8} to 10^{-4} M GA_3 (Leopold 1971). Since growth in this system depends on exogenous GA, it was hypothesized that ancymidol affected GA action or metabolism. Note that ancymidol (1 μM) inhibited abscisic acid synthesis by about 20% in a fungal system (Norman et al. 1983).

Tetcyclacis also restricts GA biosynthesis through the inhibition of *ent*-kaurene oxidation (Rademacher et al. 1983). At low concentrations (10^{-6} M or less), the growth inhibitory action of tetcyclacis on intact plants appears to be exerted primarily through reduced cell elongation (Nitsche et al. 1985). Under these conditions, inhibition was completely reversed by GA (Rademacher and Jung 1981, Raskin and Kende 1984). However, at concentrations between 10^{-6} and 10^{-4} M, tetcyclacis inhibited cell division in intact plants and in cell cultures and disrupted sterol biosynthesis in cells (Grossmann et al. 1983, 1985, Nitsche et al. 1985). The effects on cell cultures were reversible with added sterols but not with GA (Grossmann et al. 1985).

The following experiments were therefore undertaken to assess the extent to which GA treatment would prevent the expression of growth inhibition by tetcyclacis and ancymidol in the *dwarf*-5 mutant of *Zea mays* L. A GA_{4+7} mixture was used, because it promoted growth better than GA_3.

Materials and Methods

Plants were raised in controlled environment chambers (EGC, Chagrin Falls, OH) under 14 h LD (550–600 μmols^{-1} m^{-2} between 400 and 700 nm from cool white-fluorescent and incandescent lamps; 27°C, 65% RH and 350 μl l^{-1} CO_2) in vermiculite (4-in. pots) with daily application of a complete nutrient solution. Inhibitors were added approximately 11 days after sowing when the ligule of the third leaf had appeared above the subtending leaf sheath. Ancymidol (Elanco Products Co., Indianapolis) at 0.2 μM or tetcyclacis (BASF, West Germany) at 3.7 μM were prepared in 0.1% Tween-20 and applied as a root drench (100 ml per plant). A 30-μM mixture of GA_{4+7} (Abbott Laboratories, North Chicago, IL) was prepared in 0.1% Tween-20 and 5% ethanol and was pipetted (0.5 ml) directly into the whorl. Controls received the Tween-20 root drench treatment as well as the Tween-20/ethanol solution in the whorl. Plants were harvested 7 days after the initial inhibitor treatment, at which time the length of the fifth leaf sheath and the dry weight of the shoot were determined. A ruler was used to measure length to the nearest 0.25 mm. Two replicate experiments, each with 16–20 plants per treatment, yielded essentially identical results.

Results and Discussion

Visible effects of GA (increased leaf sheath lengths, longer and thinner leaves, chlorosis) were observed within 1–2 days of application, whereas 2–3 days were required to see any effect of the inhibitors (reduced shoot length). Aside from the fact that growth promotion was more dramatic than inhibition, exogenous GAs presumably required less time to reach or to affect the sites of growth regulation than did the inhibitors that were taken up through the roots. Consequently, a 1-day waiting period was inserted between inhibitor treatment and GA application to equalize approximately the onset of biological activity.

Typical results are presented in Table 1. Treatment with GA_{4+7} (30 μM) resulted in 4.31- and 1.74-fold increases in the length of the fifth leaf sheath and in shoot dry matter, respectively. The effect on leaf sheath elongation was much greater, because the sheath was still developing at the time of treatment, whereas total shoot dry matter included a substantial mass that was no longer growing and that presumably did not respond. Ancymidol (0.2 μM) caused 28% and 15% decreases in sheath length and dry matter, respectively, whereas tetcyclacis (3.7 μM) caused 41% and 25% reductions, respectively. Both effects of the inhibitors were statistically significant at the 95% confidence level.

In the presence of GA_{4+7}, ancymidol and tetcyclacis caused only slight reductions in leaf sheath length and the amount of shoot dry matter relative to the plants that received GA alone. The differences were not significant at the 95% confidence level but were observed in both replicates. They may have been caused by the delay between inhibitor and GA treatments, or they may reflect a small, residual inhibition not reversible by GA. It is important to note that plants receiving both growth retardants and GA appeared morphologically identical to those that received GA only.

Table 1. Growth inhibition of the *dwarf*-5 mutant of *Zea mays* L. by ancymidol and tetcyclacis and its prevention by GA_{4+7}.

Treatment	Leaf sheath length (cm)	Shoot dry matter (mg)
Control	4.25 ± 0.14[c]	858 ± 34[c]
GA_{4+7}	18.31 ± 0.47[d]	1,495 ± 56[d]
Ancymidol	3.06 ± 0.09[b]	726 ± 34[b]
Ancymidol + GA_{4+7}	16.67 ± 0.73[d]	1,421 ± 71[d]
Tetcyclacis	2.52 ± 0.08[a]	641 ± 23[a]
Tetcyclacis + GA_{4+7}	17.33 ± 0.75[d]	1,388 ± 79[d]

Ancymidol (0.2 μM), tetcyclacis (3.7 μM), or control treatments were added as root drenches (100 ml per plant) 11 days after sowing. Treatment was made within the first hour after lights on; watering was withheld for the remainder of the day to allow uptake. GA_{4+7} (30 μM) or control treatments were applied 24 h later. Plants were harvested 18 days after sowing. Values are the mean ± 1 SE (n = 20). Within a column, different superscripts indicate differences significant at the 95% confidence level.

The results are consistent with an action of ancymidol and tetcyclacis on GA biosynthesis in *dwarf*-5, since the inhibition can be "completely overcome" (Lang 1970). The inhibitor studies thus constitute important, independent evidence that the genetic block to GA formation in the mutant is incomplete. Final proof, however, must await improvements in analytical procedures for GAs.

We are unaware of any previous reports on the action of growth retardants on GA-deficient mutants of corn, although ancymidol (Shive and Sisler 1976) and 1-*n*-decylimidazole (Wada and Imai 1980) inhibited growth in the Tan-ginbozu dwarf mutant of rice. Inhibition by the latter compound was partially reversible by added *ent*-kaurenoic acid, suggestive of an effect on GA metabolism. The possible blockage of GA biosynthesis by chemical growth retardants must therefore be considered even when working with dwarf mutants. In fact, it may be beneficial to use plants dwarfed both genetically and chemically in the study of GA physiology, provided that the action of the chemicals can be ascribed to an inhibition of GA biosynthesis. Note, however, that growth regulator action and metabolism in plants may be qualitatively different under conditions of hormone depletion (Evans 1985). This uncertainty limits the usefulness of control data from dwarf plants.

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