Plant Physiol. (1983) 71, 15–18
0032-0889/83/71/0015/04/$00.50/0

Inhibition of Abscisic Acid Biosynthesis in *Cercospora rosicola* by Inhibitors of Gibberellin Biosynthesis and Plant Growth Retardants

Received for publication May 28, 1982 and in revised form September 2, 1982

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ABSTRACT

The fungus *Cercospora rosicola* produces abscisic acid (ABA) as a secondary metabolite. We developed a convenient system using this fungus to determine the effects of compounds on the biosynthesis of ABA. Inasmuch as ABA and the gibberellins (GAs) both arise via the isoprenoid pathway, it was of interest to determine if inhibitors of GA biosynthesis affect ABA biosynthesis. All five putative inhibitors of GA biosynthesis tested inhibited ABA biosynthesis. Several plant growth retardants with poorly understood actions in plants were also tested; of these, six inhibited ABA biosynthesis to varying degrees and two had no effect. Effects of plant growth retardants on various branches of the isoprenoid biosynthetic pathway may help to explain some of the diverse and unexpected results reported for these compounds. Knowledge that certain inhibitors of GA biosynthesis also have the ability to inhibit ABA biosynthesis in *C. rosicola* indicates the need for further studies in plants on the mode of action of these compounds.

Phytohormones act in coordinating plant growth and development through interconnecting reactions that are presently poorly understood (15). Two of these phytohormones, ABA and the GAs[1] tend to bring about opposite physiological effects. ABA has been implicated in the inhibition of growth and RNA synthesis, the promotion of senescence, abscission, and stomatal closure, and is involved in the adaptation of plants to stress conditions (34). In contrast, the GAs promote growth, overcome dormancy, and delay senescence and abscission (11, 25).

ABA, GAs, steroids, carotenoids, and higher isoprenoids share mevalonate as a common precursor. Inhibitors have been used to study isoprenoid biosynthetic pathways and some inhibitors of GA biosynthesis are used as plant growth retardants (18, 30, 35). Agriculturally important plant growth retardants, CCC and ancymidol, and experimental compounds, AMO 1618 and decylimidazole, have been shown to inhibit GA biosynthesis in *Gibberella fujikuroi* and certain higher plants (5–7, 10, 32, 33). Therefore, these compounds are considered by some to suppress plant growth in part by inhibiting endogenous GA biosynthesis (18, 30, 37). The mode action of many other plant growth retardants is poorly understood. Little is known about the effect of inhibitors of GA biosynthesis and plant growth retardants on ABA biosynthesis.

Knowledge of the regulation of GA biosynthesis and the effects of a wide variety of compounds thereon has been greatly advanced through studies using the fungus *G. fujikuroi* or isolated plant enzyme systems (11, 25, 36). We developed a convenient system using the fungus *Cercospora rosicola*, which produces ABA as a secondary metabolite (1), to test the effect of various compounds on ABA biosynthesis (2, 19, 20). No inhibitors of ABA biosynthesis had been reported until the recent finding that cytokinins inhibit ABA biosynthesis in *C. rosicola* (19). Because of the opposite physiological effects of ABA and the GAs and since both arise via the isoprenoid pathway, it is important to know whether or not inhibitors of GA biosynthesis and plant growth retardants affect ABA biosynthesis. Knowledge of the action of exogenous growth retardants on hormonal regulation and coordination of plant growth, development, and senescence should aid in understanding the effects and defining the utility of these chemicals. This paper reports the effects of several compounds on ABA biosynthesis in the fungus *C. rosicola*.

MATERIALS AND METHODS

Cultures. *Cercospora rosicola* Passerini cultures (strain 138.35) were from the Centraalbureau voor Schimmelcultures, Baarn, The Netherlands. Growth in defined medium was described previously (20). Mycelium harvested during the ABA-producing phase (6–7 d old) was collected on a screen, washed twice with sterile H_2O, and (6–10 ml) resuspended into fresh defined medium diluted 1:5 with water (60–100 ml). Test compounds in water were filter sterilized and added to the diluted media. Each compound was tested at two or more concentrations in duplicate. Resuspensions were incubated on a rotary shaker at 22 to 23°C for 48 h. After 48 h, the mycelium was collected by filtration, freeze-dried, and weighed. Compared to the controls, no alteration in mycelium dry weight or change in pH of the media was observed with the test compounds at the concentrations reported.

Test Compounds. The following chemicals were from Sigma[2]: maleic hydrazide, Polaris, and CCC. AMO 1618 was from Calbi-

[1] Nomenclature and abbreviations: GA, gibberellin; CCC, (2-chloroethyl)trimethylammonium chloride; ancymidol, α-cyclopropyl-α-(*p*-methoxyphenyl)-5-pyrimidine methyl alcohol; AMO 1618, 2′-isopropyl-4′-(trimethylammonium chloride)-5′-methylphenylpiperidine-1′-carboxylate; decylimidazole, 1-*n*-decylimidazole; maleic hydrazide, 1,2-dihydro-3,6-pyridazinedione; Polaris, *N*,*N*-bis(phosphonomethyl)glycine; Alar, succinic acid-2,2-dimethylhydrazide; morphactin, 9-hydroxy-9-fluorenecarboxylic acid; PIX, 1,1-dimethylpiperidinium chloride; DMM, 4,4-dimethylmorpholinium iodide; DCPT, 2-(3,4-dichlorophenoxy)triethylamine; Tridemorph, 2,6-dimethyl-*N*-*n*-tridecylmorpholine; dikegulac, 2,3:4,6-bis-*o*-(1-methylethylidene)-α-L-xylo-2-hexulofuranosonic acid.

[2] Reference to brand or firm name does not constitute endorsement by the United States Department of Agriculture over others of a similar nature not mentioned.

ochem-Behring Corp., and Alar and morphactin from Aldrich Chemical Co. The following compounds were gifts: ancymidol from Eli Lilly and Co. and PIX from BASF Corp. Decylimidazole was synthesized according to Wada (32). DMM was synthesized by reacting a 2-fold excess of methyl iodide with 4-methylmorpholine in anhydrous ethyl ether. The precipitate was filtered and recrystalized from 25% methanol in ethanol. DCPT was synthesized according to Scheutz and Baldwin (27). Tridemorph was synthesized by reacting *n*-tridecylbromide with an excess of 2,6-dimethylmorpholine in place of diethylamine as previously described (24) and recrystalized as the hydrochloride from isopropanol:diethyl ether (1:1).

HPLC. One ml of the culture media was removed at 0, 24, and 48 h for measurement of ABA by HPLC as previously described (22). The acidified liquid medium was passed through a C_{18} reversed-phase cartridge for cleanup, and the ABA fraction eluted with 75% methanol in 20 mM H_3PO_4. ABA was separated on a Partisil PXS 5/25 ODS column (Whatman Inc.) with 50% methanol in 20 mM H_3PO_4 at a flow rate of 1 ml/min. Samples (100 μl) were measured at 268 nm. ABA was calculated in μg/ml during the tests and in mg/g dry weight at 48 h compared with standard ABA solutions. Percent inhibition was determined by comparison with triplicate untreated controls in each experiment.

RESULTS AND DISCUSSION

The effects of the test compounds on ABA biosynthesis are shown in Table 1. The well-known inhibitors of GA biosynthesis, declimidazole, ancymidol, AMO 1618, and CCC, inhibited ABA biosynthesis in *C. rosicola.* Wada (32) found that decylimidazole inhibits the transformation of (−)-kaurene and (−)-kauren-19-ol to (−)-kaurenoic acid in GA biosynthesis of *G. fujikuroi.* About 200 μM of decylimidazole was required for complete inhibition of microbial GA biosynthesis, and 400 μM caused growth retardation in rice seedlings (32, 33). In contrast, decylimidazole is a more active inhibitor of ABA biosynthesis; complete inhibition was obtained at 100 μM. ABA production by the fungus and the inhibition of ABA by decylimidazole at 48 h is shown in Figure 1.

Ancymidol was the second most active inhibitor of ABA biosynthesis. Coolbaugh *et al.* (6) reported that ancymidol is a highly specific inhibitor of three oxidative reactions in the GA biosynthetic pathway in higher plants but that it does not inhibit GA biosynthesis in the fungus *Fusarium moniliforme* (*G. fujikuroi*). Ancymidol was the most potent inhibitor of the GA pathway in plants with activity at 2 nM. Ancymidol reduced endogenous GA content of morning glory plants (31) and beans (29) but did not inhibit sterol biosynthesis in beans.

Table I. *Effects of Compounds on ABA Biosynthesis by C. rosicola*

Percentage of inhibition was calculated as follows: 100 − (ABA mg/g dry weight mycelia of treatment ÷ the mean of ABA mg/g dry weight mycelia of three untreated controls) × 100. The mean ± SD of a typical set of controls was 3.92 ± 0.38 mg ABA/g dry weight mycelia.

Compounds Tested	Inhibition at Following Concn. (μM)					
	1,000	500	100	50	10	1
			%			
Decylimidazole			100	99	77	29
Ancymidol			82	70	29	18
Tridemorph			77	63	30	
AMO 1618			77	54	28	27
DCPT		91	35	7		
CCC	59	39	38	23	25	0
PIX		41	34			
DMM		40	28			
Maleic hydrazide		79	20	16		
Dikegulac		21	31			
Morphactin		34	0	7		
Alar	4		2			
Polaris	17		0			

AMO 1618 prevents the conversion of mevalonate into (−)-kaurene in plant and microbial GA biosynthesis (7) and inhibits the conversion of acetate and mevalonate to sterols (8, 9). AMO 1618 inhibited ABA biosynthesis in *C. rosicola.* It was twice as active as CCC at 100 μM and more active at lower concentrations. Milborrow (17) found that 10 μM of AMO 1618 did not affect incorporation of labeled mevalonate into ABA in avocado mesocarp. On the other hand, 1,000 μM caused a 3-fold enhancement. This enhancement was attributed to the inhibition of triterpene and steroid synthesis, thereby making more mevalonate available for carotenoid and ABA biosynthesis. However, Milborrow thought that the rate of synthesis might have actually been reduced. Whether the action of AMO 1618 on microbial ABA biosynthesis is the same as that in plants remains to be determined. AMO 1618 has a narrow spectrum of activity in plants (4), and other plant tissues might respond differently than the avocado.

CCC inhibits the conversion of geranylgeranyl pyrophosphate to (−)-kaurene in GA biosynthesis, and Wada (32) determined that about 1,260 μM of CCC inhibited microbial GA biosynthesis by 80%. We obtained about 60% inhibition of ABA biosynthesis with 1,000 μM of CCC. CCC was active over a wide concentration range and inhibition of ABA biosynthesis did not drop as rapidly with decreasing concentration as with the other compounds tested. CCC is one of the most widely used plant growth regulators worldwide. Its action as a height shortener has been attributed to the inhibition of GA biosynthesis (18, 26, 35, 37). Our results show that CCC inhibits ABA biosynthesis equally as well. Milborrow (17) used the avocado mesocarp system to test CCC and other inhibitors of GA and carotenoid biosynthesis such as Phosphon D, diphenylamine, and 3-amino-1,2,4-triazole and found that the incorporation of labeled mevalonate into ABA was not affected. Its effect on ABA biosynthesis in other plant tissues warrants study to determine whether the action of CCC on ABA biosynthesis in plants is the same as that in *C. rosicola.*

The plant growth retardant, Tridemorph (12), has been reported to inhibit cycloeucalenol-obtusifoliol isomerase in sterol biosynthesis in bramble cells (28). The action of Tridemorph on GA biosynthesis is not known. Tridemorph was as active as AMO 1618 in inhibiting ABA biosynthesis in *C. rosicola.*

Substituted 2-phenoxyethylamines were first found to have biological activity in plants by Jones *et al.* (13). DCPT is an experimental compound shown to induce polyisoprenoid rubber accumulation in guayule (38) but has not been reported to retard plant growth. DCPT inhibited GA biosynthesis 33% at 100 μM in *G. fujikuroi* (10). ABA biosynthesis in *C. rosicola* was inhibited to the same extent at 100 μM. The concentration range for inhibition of ABA biosynthesis is narrow compared with CCC and AMO 1618.

PIX is used on cotton plants to decrease plant height and length of lateral branches (18). Its effect on GA biosynthesis is not known. DMM is a plant growth retardant that may block GA biosynthesis and accelerate auxin catabolism (14). These two compounds inhibited ABA biosynthesis to about the same extent as CCC and DCPT at 100 μM.

Maleic hydrazide is another major plant growth retardant used in agriculture. The mode of action of maleic hydrazide, although studied for 30 years, is not well understood (30). It does not affect GA biosynthesis in *G. fujikuroi* (10). Alar, also a major agricultural plant growth retardant, has effects similar to those of CCC and is thought to be involved in late stages of GA biosynthesis (30, 35) although it has no effect on GA biosynthesis in *G. fujikuori* (10). Maleic hydrazide inhibited ABA biosynthesis at high concentra-

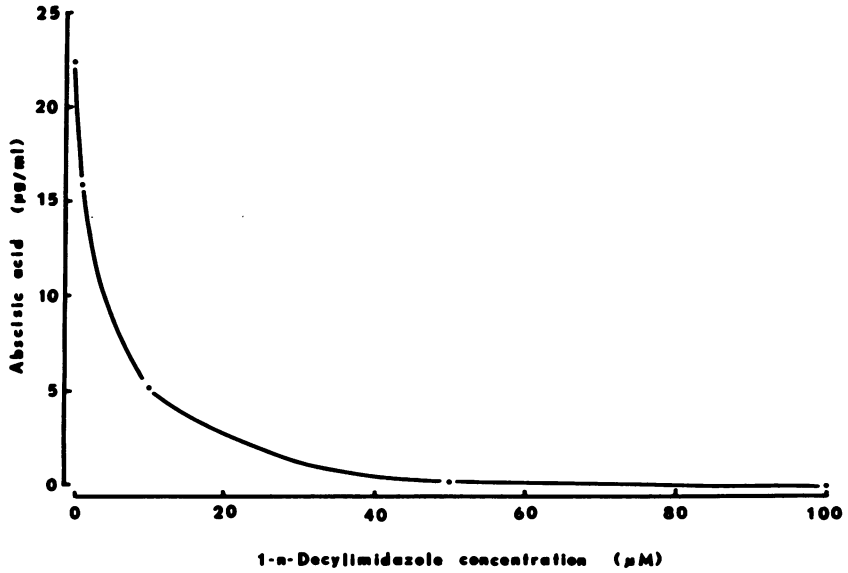

FIG. 1. Decylimidazole concentration-dependent inhibition of ABA biosynthesis by *C. rosicola.*

tions and Alar had no effect.

Polaris is an effective sugar-cane ripener. The mode of action of Polaris is poorly understood, but its ripening activity may be due to a combination of growth inhibition, increased translocation, and reduced acid invertase activity (16). The effect of Polaris on GA biosynthesis is not known. Polaris did not affect ABA biosynthesis by the fungus.

Dikegulac is used as a pinching agent and as a growth retardant. Its action is antagonized by exogenous GAs similarly to the effect of GA on the action of CCC. This leads to the conclusion that dikegulac might inhibit GA biosynthesis although this has not been demonstrated (3). Dikegulac reduced ABA biosynthesis, but the effect was not concentration dependent, indicating an indirect effect on ABA biosynthesis.

Derivatives of fluorene-9-carboxylic acid (morphactins) have many physiological effects and are used to retard growth of grasses and weeds (26, 30, 37). Parups (23) found that morphactin treatment of beans caused the endogenous levels of IAA, ABA, GA_1, and GA_9 in the roots to decrease with increasing amounts of morphactin. The ABA content was suppressed more than that of the auxins resulting in an imbalance which disturbed growth. Other auxins, GA_3 and GA_4, were not affected. In *C. rosicola*, morphactin inhibited ABA biosynthesis somewhat at 500 μM but had no effect at lower concentrations.

Many studies have shown that plants respond differently to plant growth retardants. For example, of 88 ornamental species, 21 respond to CCC, 68 respond to ancymidol, and only five respond to AMO 1618 (4). Incorporation of labeled mevalonate into ABA in avocado tissue was enhanced by AMO 1618 and was not affected by CCC (17). CCC is an effective height shortener in wheat but gives variable responses in rye and is ineffective in barley. Even within single varieties, responses depend on the physiological stage of development and hormonal balance. Height shortening has been attributed to the inhibition of GA biosynthesis by CCC (18, 30, 35, 37). In contrast, CCC can also produce a marked delay in wilting and senescence in some plants under stress conditions, a response which cannot be attributed to inhibition of GA biosynthesis (37). The influence of plant growth retardants on various branches of the isoprenoid biosynthetic pathway, especially those leading to ABA and the GAs which have opposite hormonal activities in plants, may help explain some of the diverse and unexpected results reported for these compounds. The finding that certain inhibitors of GA biosynthesis also have the ability to inhibit ABA biosynthesis in *C. rosicola* indicates the need for further studies in plants on the mode of action of these compounds.

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