Phytochemistry, Vol. 25, No. 1, pp. 93–95, 1986.
Printed in Great Britain.

0031-9422/86 $3.00 + 0.00
Pergamon Press Ltd.

CATECHINS AS GERMINATION AND GROWTH INHIBITORS IN *LESPEDEZA* SEEDS

J. GEORGE BUTA* and WILLIAM R. LUSBY†

Plant Hormone Laboratory, Plant Physiology Institute* and Insect Physiology Laboratory, Plant Protection Institute†, ARS, U.S. Department of Agriculture, Beltsville, MD 20705, U.S.A.

(*Received* 27 *April* 1985)

Key Word Index—*Lespedeza bicolor*; *L. cuneata*; *L. stipulacea*; Leguminosae; catechin; epicatechin; seed germination; growth inhibition.

Abstract—The germination and growth inhibiting compounds found in *Lespedeza bicolor*, *L. cuneata* and *L. stipulacea* seeds have been shown to be catechin and epicatechin. Differences in time of germination and amount of early seedling growth (*L. stip.* > *L. cun.* = *L. bic.*) were inversely related to the quantities of these polyphenols found in the seeds.

INTRODUCTION

During an investigation of the growth inhibitors isolated from *Lespedeza* seeds, it was observed that *Lespedeza stipulacea* (Korean) seeds germinated in two or three days while the seeds of *Lespedeza cuneata* (sericea) and *L. bicolor* (shrub) required at least six days for germination. A possible explanation for this is the presence of water-soluble inhibitors in the germinating seed and this was confirmed by bioassay. These inhibitors appeared to affect both the rate of germination and subsequent young seedling growth of *Lespedeza* seeds. In this report, we describe the isolation and identification of the growth inhibitors present in the seeds of the three *Lespedeza* species. The quantities of these inhibitory compounds isolated from the seeds are correlated with the time required for germination.

RESULTS

Seeds of the three species of *Lespedeza* were tested for differences in rate of germination and subsequent seedling root growth. It was confirmed that the *Lespedeza stipulacea* seeds germinated more rapidly (70% after 3 days; 90% at 7 days) than did those of *L. bicolor* (10% and 30%) and *L. cuneata* (20% and 80%). It was also observed that root growth of those *L. bicolor* (2.0 mm) and *L. cuneata* (16.6 mm) seedlings that had germinated after 7 days was inhibited compared to that of the *L. stipulacea* seedlings (35.7 mm).

Seed of the three species were immersed in water for 16 hr and the resulting solutions were used in lettuce seed germination bioassays to determine whether growth inhibitors were being leached from the seeds. The levels of inhibitors in solutions derived from *L. bicolor* and *L. cuneata* seeds being greater then that obtained from *L. stipulacea* seeds (Table 1). If the leachate from *L. cuneata* seeds was added to *L. stipulacea* seeds, a marked reduction in seedling growth was observed (Table 1).

Isolation of the inhibitory compound leached from *Lespedeza* seeds was performed using the lettuce seed bioassay to locate the growth inhibitory activity. (+)-Catechin and (−)-epicatechin were identified as the inhibitory compounds present in the three species of *Lespedeza* seeds after analysis by TLC, NMR, MS and HPLC. The quantities of catechin and epicatechin readily leached from the seeds in sixteen hours are compared to the total content of these compounds in the seeds

Table 1. Effects of *Lespedeza* seed leachates on the root growth of seedlings

	Lettuce (mm)†			
Concentration*	0	0.01 ×	0.1 ×	1 ×
Lespedeza bicolor	55.7ab	54.2ab	53.6ab	18.3c
Lespedeza cuneata		52.0ab	47.3ab	22.6c
Lepedeza stipulacea		60.8a	58.0a	34.3bc
	Lespedeza stipulacea (mm)			
Lespedeza cuneata	27.3	27.4	18.1	6.9

*Concentration of the leachate is the result of 16 hr of immersion of 5.0 g of seeds in 20 ml H_2O. Root growth was measured after six days.

†Means separated by Duncan's multiple range test, 5% level.

Table 2. Catechins in *Lespedeza* seeds

	(+)-Catechin		(−)-Epicatechin	
	16 hr leachate* (μg/g)	total†	16 hr leachate (μg/g)	total
Lespedeza bicolor	60 (0.5)	400	200 (1.7)	2000
Lespedeza cuneata	120 (1.0)	200	400 (3.4)	5000
Lespedeza stipulacea	2 (0.02)	5	30 (0.3)	50

*Quantity present in aqueous leachate after 16 hr of immersion of 5.0 g seeds in 20 ml H_2O. Numbers in parentheses indicate molar concentration $\times 10^{-4}$.
†Quantity present in methanolic extract of ground seeds.

(Table 2). The quantities of these compounds found in the seeds of the three species are inversely related to the rates of germination and subsequent seedling growth that were measured. The growth regulating activity of catechin and epicatechin (pure compounds) in the lettuce seed assay and an assay using *Lespedeza stipulacea* seeds is shown in Table 3. The total concentration of catechin and epicatechin leached from *L. cuneata* seeds in sixteen hours is 4.4×10^{-4} M which can account for the growth inhibiting activity observed in the lettuce bioassay based on a comparison with the activity of the pure compounds.

The more rapid rate of seed germination and seedling growth of *Lespedeza stipulacea* may be attributed to the relatively low levels of catechin and epicatechin present in the seeds. In contrast, the seed germination and early seedling growth of the other two *Lespedeza* species is delayed by the relatively high levels of these inhibitory compounds found in the seeds. Longer periods of time are required before the quantities of catechin and epicatechin are reduced to non-inhibitory levels in those seeds.

Catechin and epicatechin have not been reported previously to be present in *Lespedeza* seeds or in other parts of the plants [1, 2]. Catechin was reported to be an endogenous regulator of seed germination and seedling growth in peaches [3]. Since an inhibition of the peroxidative oxidation of indole-3-acetic acid was found, a role for catechin in the control of hormone metabolism in peach seeds was suggested. Similar polyphenols have been found to be inhibitors of sulphydryl transferase [4] and phosphorylase [5] enzymes so that multiple sites of action for catechin and epicatechin may be possible.

Therefore, while the sites of action for these compounds may not be known, the differences in quantities of catechin and epicatechin present in *Lespedeza* seeds appear to account for the differences in rates of germination and early seedling growth found for these three species.

EXPERIMENTAL

Assay. Seeds of lettuce *Lactuca sativa* cv. Grand Rapids or the various *Lespedeza* seeds were used in four-segmented Petri dishes and the method of Tang and Young was followed [6].

Isolation and identification of inhibitors. Seeds of the various *Lespedeza* species (5 g) were immersed in 20 ml H_2O and kept at room temp. for 16 hr. After filtration and washing of the seeds, the combined solns were extracted twice with EtOAc (20 ml). The concd extracts were applied to silica (0.25 cm thick) TLC plates (5 × 20 cm) and developed with CH_2Cl_2–MeOH (4:1). A band at R_f 0.45 after extraction showed inhibitory activity with the lettuce seed assay. The compound(s), R_f 0.45, had UV λ_{max}^{MeOH} nm: 281 with IR and ^{1}H NMR also indicating a flavonoid moiety. The compounds were identified as a mixture of catechin, *m/z* 290 $[M]^+$, and epicatechin, *m/z* 290 $[M]^+$, by EIMS probe 70 eV and CIMS (NH_3). Confirmation was done by reverse phase HPLC using a 10 μm polystyrene analytical column and a gradient solvent system (MeCN–0.006 N HCl) at 1.3 ml/min [7]. Catechin (R_t 10.92) and epicatechin (R_t 13.24) were separated and quantified in the *Lespedeza* seed leachates by this method. Methanolic extraction of the catechins from the ground *Lespedeza* seeds was done at 10° and analysis of the compounds was done by HPLC.

Table 3. Effects of catechins on root growth of seedlings

Concentration (M, × 10^4)	0	1	5	10	50
			Lettuce (mm)		
(+)-Catechin	54.1a*	55.5a	21.1bc	17.0bc	0.0d
(−)-Epicatechin		52.1a	25.9b	13.5c	0.0d
			Lespedeza stipulacea (mm)		
(+)-Catechin	21.4		21.6		5.0
(−)-Epicatechin			15.1		5.0

*Means separated by Duncan's multiple range test, 5% level. Root lengths measured after six days.

Acknowledgement—The authors wish to thank Dr. Arden N. Reed for assistance with statistical analysis.

REFERENCES

1. Glyzin, V. I., Bankovskii, A. I. Zhurba, O. V. and Sheichenko, V. I. (1970) *Chem. Nat. Cmpds* **6**, 473.
2. Wagner, H., Iyenger, M. A. and Horhammer, L. (1972) *Phytochemistry* **11**, 1518.
3. Ritzert, R. W., Hemphill, D. D., Sell, H. M. and Bukovac, M. J. (1972) *J. Am. Soc. Hort. Sci.* **97**, 48.
4. Das, M., Bichers, D. R. and Mukhtar, H. (1984) *Biochem. Biophys. Res. Commun.* **120**, 427.
5. Koeppe, D. M. and Miller, R. E. (1974) *Plant Physiol.* **54**, 374.
6. Tang, C. S. and Young, C. C. (1982) *Plant Physiol.* **69**, 155.
7. Buta, J. G. (1984) *J. Chromatogr.* **295**, 506.