

AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D. C. 20523
BIBLIOGRAPHIC INPUT SHEET

FOR AID USE ONLY

1. SUBJECT CLASSIFICATION	A. PRIMARY Agriculture	AF22-0000-0000
	B. SECONDARY Soil chemistry and physics	
2. TITLE AND SUBTITLE Ultrarapid extraction of insecticides from soil using a new ultrasonic technique		
3. AUTHOR(S) Johnson, R.E.; Starr, R.I.		
4. DOCUMENT DATE 1972	5. NUMBER OF PAGES 4p.	6. ARC NUMBER ARC
7. REFERENCE ORGANIZATION NAME AND ADDRESS Interior		
8. SUPPLEMENTARY NOTES (Sponsoring Organization, Publisher, Availability) (In J. of agr. food chemistry, v.20, no.1, p.48-51)		
9. ABSTRACT		

10. CONTROL NUMBER PN-RAA-616	11. PRICE OF DOCUMENT
12. DESCRIPTORS Extraction Insecticides Polytron? Ultrasonic tests	13. PROJECT NUMBER
	14. CONTRACT NUMBER PASA RA(ID)1-67 Res.
	15. TYPE OF DOCUMENT

Ultrarapid Extraction of Insecticides from Soil Using a New Ultrasonic Technique

Richard E. Johnsen* and Robert I. Starr¹

The Polytron, a high specific intensity ultrasonic generator, was used to extract insecticide residues from various soils. This system was compared with a second ultrasonic source and with Soxhlet, roller, and blender extraction procedures. The solvent of choice was acetone. Soil moisture, type, and or-

ganic matter content were not critical factors in affecting quantitative recovery. Extraction for only 30 sec gave generally better recovery values than did other methods, including 8 hr of Soxhlet extraction.

The soil is a principle reservoir of environmental pesticides, particularly for the organochlorine insecticides. The persistence of these insecticides has aroused considerable interest in recent years, as evidenced by extensive soil monitoring activity at local and national levels (Duffy and Wong, 1967; Fahey *et al.*, 1965; Seal *et al.*, 1967; Stevens *et al.*, 1970; Trautman *et al.*, 1968).

Pesticide analysts are seeking continuously to improve procedures for the determination of residues primarily by reducing analysis time and increasing accuracy and sensitivity. The extraction of residues from soil is as important as their determination since residues not extracted are not measured. Since soils are highly variable and complex, there is no standard extraction method. Chiba (1969) summarized and discussed the available data on extraction of organochlorine insecticides from soil. According to Burchfield *et al.* (1965), residue extraction from soils where aging has followed application is difficult, due in part to binding by soil micelles and by the retention of residues by soil flora and fauna. They state that current extraction methods make no provision for rupturing cells and liberating bound residues.

In the recent past, ultrasonic energy has been used in the extraction of various materials from plants, animals, and microorganisms (Frye, 1958) and has been used widely to facilitate the extraction of commercially important substances (Babikov, 1960; Schroder, 1953). The initial use of ultrasonic energy as a means to extract organochlorine residues from soil was reported and later refined by Johnsen and Starr (1967, 1970).

The purpose of this study was to develop a rapid, reliable, and sensitive soil extraction method using the Polytron, a high specific intensity ultrasonic generator. This method was compared with other extraction methods and various experimental conditions were tested for their effect on extraction efficiency to determine the optimum and limiting conditions for use of the Polytron.

MATERIALS AND METHODS

Insecticides. Analytical grade insecticide standards were used. Dieldrin was supplied kindly by Shell Chemical Co., heptachlor epoxide by Velsicol Chemical Corp., *p,p'*-DDT,

o,p'-DDT, and *p,p'*-DDE by Geigy Chemical Co., and lindane, γ -chlordane, and *p,p'*-methoxychlor by the Food and Drug Administration. Dieldrin and heptachlor epoxide were used most commonly for the reasons reported previously (Johnsen and Starr, 1967). Duplicated solutions of the insecticides were made in both acetone and benzene, the former for soil treatment and the latter as an analytical standard.

Preparation of Soil Samples. The characteristics of the soils presented in Table I were determined by the Colorado State University Soil Testing Laboratory. Since clay content is one of the most important soil factors affecting adsorption of organochlorine insecticides (Harris, 1966), soil No. 2 was the predominant soil used in this study. Various standard clay minerals used in one experiment were obtained from Ward's Natural Science Establishment, Rochester, N.Y., and were ground to pass an 18-mesh screen prior to use. The air-dried soil samples (50.0 g oven-dried basis) in 0.5-l. French square bottles were treated individually by pipeting onto the soil surface 10 ml of an acetone solution of the insecticide. The bottle then was rotated gently to mix the soil. The insecticides used were usually 100 μ g of both heptachlor epoxide and dieldrin unless stated otherwise and the soil was aged for at least 1 month before extraction. Additional details on the preparation of field-treated soils and the treatment, handling, and storage of the other soil samples have been described (Johnsen and Starr, 1970).

Solvents. All the solvents used were redistilled in glass prior to use. The primary extraction solvent was acetone; others used were hexane-acetone, chloroform-methanol (both 1:1 v/v), benzene-methanol (2:1 v/v), ethanol and petroleum ether (bp 30-60°C).

Extraction Procedures. The soil samples after aging usually were extracted ultrasonically with the Polytron (Model PT-20ST, Brinkmann Instruments, Westbury, N.Y.), an improved version of the Ultra-Turrax. The generator which is immersed in the sample was equipped with a saw tooth cutting head which aided in reducing soil particles and aggregates to a very fine "powder." This model has a pulse-frequency maximum of 9400 cps (9.4 KHz) and a maximum generator resonator speed of 19500 rpm. Motor speed, and thereby effective frequency, amplitude, and specific intensity, is controlled by a continuously variable rheostat. A more detailed description of the Polytron, other models, and their applications can be found in the company bulletin (Brinkmann Instruments, 1969). Prior to extraction, the soil samples were saturated with water (30-40 ml) unless otherwise

Department of Entomology, Colorado State University, Fort Collins, Colorado 80521.

¹ Present address: Bureau of Sport Fisheries and Wildlife, Wildlife Research Center, Denver Federal Center, Denver, Colorado 80225.

Table I. Physical Characteristics of Soils Studied

Soil no.	% Soil moisture ^a	pH	% Organic matter	Inorganic separates (%)			CEC ^b	Texture
				Sand	Silt	Clay		
1	12.3	5.7	34.6	55	32	13	118	M
2	8.2	7.9	4.4	5.5	40	54.5	35.0	C
3	3.4	8.1	1.5	54	28	18	14.4	S-L
4	3.8	7.2	3.1	68	24	8	12.8	S-L
5	2.2	8.3	0.8	64	12	24	11.8	S-C-L
6	3.6	6.5	1.5	39	45	16		L
7	4.6	7.5	3.9	31	30	39		C-L
8	1.8	7.2	2.3	68	12	20		S-L
9	2.0	7.4	3.6	8	52	42		Si-C

^a In air-dried soil. ^b Cation exchange capacity in mequiv/100 g. ^c M, C, L, S, and Si represent muck, clay, loam, sandy, and silty, respectively.

Table II. Comparison of Solvents for Extraction from a Clay Soil

Extraction method	Solvent	Mean % recovery and deviation from mean	
		Heptachlor epoxide	Dieldrin
Polytron	Benzene-methanol	86.9 ± 2.0	78.9 ± 2.8
Polytron	Ethanol	96.0 ± 0.0	93.6 ± 1.6
Polytron	Hexane-acetone	94.5 ^a	92.3 ^a
Polytron	Petroleum ether	19.9 ^b	15.0 ^b
Polytron	Acetone	100.3 ± 0.8	99.1 ± 0.8
Soxhlet	Hexane-acetone	95.6 ± 3.7	92.3 ± 4.7

^a Duplicate sample lost during cleanup. ^b Single sample.

Table III. Effect of Soil Moisture Level on Extraction from a Clay Soil

Extraction method	ml H ₂ O added	Mean % recovery and deviation from mean	
		Heptachlor epoxide	Dieldrin
Polytron	0	94.5 ± 0.3	91.4 ± 1.3
Polytron	5	91.8 ± 1.6	86.6 ± 0.4
Polytron	10	97.6 ± 1.8	91.8 ± 3.3
Polytron	15	96.3 ± 1.2	92.2 ± 3.0
Polytron	30	97.3 ± 3.1	93.7 ± 2.5
Soxhlet	30	95.6 ± 0.8	87.8 ± 2.8

Table IV. Comparison of Extraction Methods and Soil Moisture Level on Recovery from a Clay Soil

Extraction method	Soil moisture	Mean % recovery and deviation from mean	
		Heptachlor epoxide	Dieldrin
Polytron	Dry	97.3 ± 1.8	96.3 ± 1.4
	Wet	100.0 ± 0.2	98.4 ± 0.2
Ultrasound	Dry	97.6 ± 0.4	98.3 ± 0.0
	Wet	99.3 ± 0.1	96.0 ± 1.1
Blender	Dry	84.2 ± 2.7	79.3 ± 2.7
	Wet	95.2 ± 0.6	93.1 ± 0.3
Roller	Dry	87.8 ± 1.1	85.8 ± 0.9
	Wet	93.7 ± 0.1	89.7 ± 0.1
Soxhlet	Dry	86.5 ^a	82.0 ^a
	Wet	99.8 ± 1.9	97.0 ± 2.4

^a One sample lost during cleanup.

Table V. Effect of Extraction Time on Recovery from a Clay Soil

Extraction time, sec	Mean % recovery and deviation from mean	
	Heptachlor epoxide	Dieldrin
15	100.1 ± 1.3	100.5 ± 1.0
30	100.0 ± 0.2	100.5 ± 0.4
60	95.5 ± 2.4	97.7 ± 0.5

Table VI. Extraction of Various Insecticides from a Clay Soil

Insecticide	Mean % recovery and deviation from mean
Heptachlor epoxide	97.6 ± 1.6
Dieldrin	96.4 ± 1.3
<i>o,p'</i> -DDT	99.5 ± 0.7
<i>p,p'</i> -DDT	99.3 ± 0.5
<i>p,p'</i> -DDE	103.8 ± 3.1
γ -Chlordane	93.6 ± 4.0
Lindane	99.1 ± 2.0
<i>p,p'</i> -Methoxychlor	93.1 ± 4.2

Table VII. Effect of Insecticide Concentration on Recovery from a Clay Soil

μ g Added to soil	Mean % recovery and deviation from mean	
	Heptachlor epoxide	Dieldrin
10	96.0 ± 1.9	96.3 ± 2.2
20	94.5 ± 1.6	98.6 ± 0.6
40	96.5 ± 0.2	97.6 ± 0.7
60	96.1 ± 0.2	95.7 ± 0.2
80	96.9 ± 0.8	97.9 ± 1.5

noted. To the sample was added 200 ml of solvent, acetone, unless stated otherwise. With the Polytron generator immersed in the sample, the soil was extracted for 30 sec at maximum power. This method was compared to a second ultrasonic method and roller, blender, and Soxhlet methods; a description and the details of extraction with these methods have been published (Johnsen and Starr, 1967, 1970). The bottles containing the treated soil samples were selected at random and analyzed in duplicate to obtain a measure of analytical variation.

Cleanup of Extracts and Gas Chromatographic Analyses. The extracted soil and solvent were filtered through Whatman No. 42 filter paper on a Buchner funnel under partial vacuum. The filter paper and the soil cake were rinsed three times with the extracting solvent. The combined filtrate was subjected to cleanup and gas chromatographic analyses as described (Johnsen and Starr, 1967, 1970).

RESULTS AND DISCUSSION

With a new extraction method, it is desirable to study the influence of specific factors on the analytical procedure. In this study a number of such factors were evaluated which included solvents, soil moisture, ultrasonic effect, comparison with other methods, extraction time, insecticide concentration, soil and clay type, insecticide type, and effect of wet and dry cycles. The results are presented largely in Tables II-XII.

Table II indicates that acetone gave the highest recovery and was better than the 8 hr Soxhlet extraction used for comparison. Acetone was selected as the solvent of choice

Table VIII. Effect of Soil Type on Recovery

Soil no.	Soil type	Mean % recovery and deviation from mean	
		Heptachlor epoxide	Dieldrin
2	Clay	96.9 ± 0.7	95.0 ± 0.1
3	Sandy-loam	99.7 ± 2.4	100.5 ± 1.8
4	Sandy-loam	97.7 ± 3.8	96.6 ± 4.6
5	Sandy-clay-loam	95.3 ± 4.0	96.9 ± 4.7

Table IX. Comparison of Extraction Methods using a Muck Soil

Extraction method	Mean % recovery and deviation from mean	
	Heptachlor epoxide	Dieldrin
Polytron	97.0 ± 0.1	96.7 ± 0.8
Ultrasound	93.5 ± 0.3	95.4 ± 0.4
Blendor	92.4 ± 0.4	89.4 ± 0.5
Roller	92.9 ± 1.5	88.8 ± 0.6
Soxhlet	93.0 ± 1.4	90.6 ± 0.6

Table X. Extraction from Various Standard Mineral Clays

Clay sample	Sample moisture	Mean % recovery and deviation from mean	
		Heptachlor epoxide	Dieldrin
Kaolinite #4	Wet	100.0 ± 1.4	99.5 ± 2.7
Metabentonite #11	Wet	99.0 ± 1.6	99.3 ± 1.1
Pyrophyllite #48	Wet	99.5 ± 0.1	98.6 ± 0.6
Montmorillonite #26	Wet	73.7 ± 1.8	70.0 ± 2.0
Montmorillonite #26 ^a	Dry	93.7 ± 0.4	95.9 ± 0.7

^a This portion repeated due to poor recovery.

Table XI. Effect of Alternate Wetting and Drying of Soils on Recovery using Various Extraction Methods

Soil type	Extraction method	% Recovery and deviation from mean	
		Heptachlor epoxide	Dieldrin
Clay	Polytron	93.8 ± 0.3	95.8 ± 1.6
Clay	Ultrasound	95.0 ± 0.9	97.5 ± 0.1
Clay	Soxhlet	86.7 ± 0.6	90.0 ± 0.3
Clay	Roller	91.3 ± 0.5	95.8 ± 0.6
Clay	Blendor	89.5 ± 2.5	91.9 ± 3.1
Muck	Polytron	93.5 ± 0.4	95.5 ± 1.1
Muck	Ultrasound	92.4 ± 0.8	95.1 ± 0.3
Muck	Soxhlet	95.7 ± 0.1	99.2 ± 0.1

because it also was the easiest solvent to work with. Due to the wet soil the hydrophobic petroleum ether gave poor recovery and with the binary solvents a separation of phases occurred. The samples extracted with the binary solvents and ethanol were difficult to filter due to the fines produced which resulted in emulsions.

Table XII. Extraction of Field-Treated Soils Containing Aged DDT Residues using Various Extraction Methods

Soil no.	Soil type	Extraction method	Ppm metabolites and deviation from mean			Total ppm
			<i>p,p'</i> -DDT	<i>o,p'</i> -DDT	<i>p,p'</i> -DDE	
7	Clay-loam	Polytron	19.04 ± 0.40	5.11 ± 0.05	4.55 ± 0.05	28.70
7	Clay-loam	Ultrasound	17.83 ± 0.72	4.77 ± 0.11	4.36 ± 0.10	26.96
7	Clay-loam	Soxhlet	18.43 ± 0.65	5.04 ± 0.04	4.77 ± 0.14	28.24
8	Sandy-loam	Polytron	1.49 ± 0.06	0.40 ± 0.02	0.41 ± 0.02	2.30
8	Sandy-loam	Soxhlet	1.48 ± 0.13	0.39 ± 0.03	0.38 ± 0.01	2.25
9	Silty-clay	Polytron	2.10 ± 0.01	0.68 ± 0.00	1.36 ± 0.01	4.14
9	Silty-clay	Ultrasound	1.99 ± 0.01	0.62 ± 0.00	1.38 ± 0.02	3.99
9	Silty-clay	Soxhlet	1.91 ± 0.05	0.59 ± 0.01	1.37 ± 0.03	3.87
9	Silty-clay	Soxhlet ^a	1.40 ± 0.01	0.43 ± 0.02	1.01 ± 0.01	2.84

^a Used 1:1 chloroform-methanol as solvent.

Table III presents the effects of soil moisture on recovery using acetone. It is apparent that soil moisture is not a critical parameter for good recovery. The best recovery overall was obtained at the 30-ml water level which also was the soil saturation level for the clay soil. For future experiments, then, the soil was deactivated by the addition of water to the saturation level prior to extraction.

Since ultrasound has been used to affect chemical changes in various chemical solutions (Brown and Goodman, 1965) it was desirable to determine if exposure to ultrasonic waves would have any effect on the insecticides used. Benzene solutions (100 ml) containing 100 µg of both heptachlor epoxide and dieldrin were subjected to extraction times of 15, 30, 60, and 120 sec using the Polytron at maximum power. An identical experiment was performed with aldrin and heptachlor, the parent compounds of the epoxides dieldrin and heptachlor epoxide, respectively, except they were subjected to only a 60-sec extraction. The recoveries were all quantitative and there was no evidence of any breakdown or alteration in the four chemicals. Considerably longer periods of sonication usually are required to affect any changes.

Table IV compares the recovery values from air-dry or wet soil extracted by five different methods. It is evident that for the latter three methods, a wet soil gave better recovery than dry soil. There was little difference between the two ultrasonic methods, although the Polytron with wet soil gave the highest recovery values.

Since the 30-sec extraction time used up to this point was selected from company literature describing Polytron applications, an experiment was conducted to determine the effect of extraction time on recovery. Table V indicates that a 30-sec extraction was adequate for quantitative recovery.

Up to this point only the cyclodiene compounds heptachlor epoxide and dieldrin were used. Table VI presents the results of Polytron extraction with six additional organochlorine insecticides, indicating very good recovery for each of them. There were no indications of any metabolism of these compounds, probably due to the soils being in an air-dry state during aging.

Since 100 µg had been used in the prior experiments, it was desirable to determine the effect of initial concentration on recovery. Table VII shows that recovery values were excellent, indicating that concentration, at least within the narrow range used here, was not a limiting factor in obtaining quantitative recovery.

The Polytron can be obtained with generator resonators equipped with and without sawtooth cutting heads. An experiment was conducted to determine their effect on recovery. Although both cutting heads gave excellent recovery, the sawtooth equipped resonator was slightly

better, possibly because it aided in the breakdown of soil particles and therefore allowed better contact between solvent and soil.

Tables VIII-X are similar in scope in that they measure the effect of soil type on recovery. Table IX is identical to Table IV except that a muck soil was extracted and then only in the saturated state. It is evident (Tables VIII and IX) that soil type had little effect on recovery and that with a muck soil the Polytron extraction method was best. Since there are different types of mineral clays found in soils in different geographical locations, various pure standard clays were treated, aged, and extracted. Table X shows that recoveries were excellent except for montmorillonite which, upon addition of water, swelled greatly to a gel-like consistency that was difficult to filter and resulted in low recoveries. This portion was repeated and extracted in the dry state with satisfactory results.

Under field conditions, insecticides in soils would be subjected to cycles of alternate wetting and drying which should tend to "lock-in" residues into the lattice structure of the soil. To test this effect on recovery, a clay soil and muck soil were treated with 50 μg of both insecticides. After aging for only 2 days, the samples were placed in a drying oven at 50° C for 1 week. Two weeks after treatment the samples were resaturated and redried. After each cycle the bottles were loosely capped and stored in the dark. The samples, after two wet-dry cycles, were resaturated and extracted 4 weeks after treatment. The results in Table XI indicate that very little, if any, insecticide was lost by volatilization. With the clay soil, the ultrasonic cleaner was slightly better than the Polytron, with the Soxhlet giving the lowest recovery values. However, with the muck soil, the Soxhlet was slightly better than the other methods. Recovery values were very satisfactory for the clay soil with the ultrasonic methods and for all three methods with the muck soil.

The last experiment involved the comparative extraction of three field-treated soils known to contain weathered DDT residues. The results in Table XII, given in parts per million (ppm), for each of the three soils indicate that the Polytron gave the highest total recovery in ppm than did the other methods, although the differences appear small. Since chloroform-methanol has been used as an extraction solvent by some workers for soil (Saha, 1968) and plants (Mumma *et al.*, 1966), it was included here as a comparison to acetone for Soxhlet extraction; it gave considerably less recovery of DDT than did acetone.

Recovery of 100- μg amounts of both insecticides carried through the cleanup procedure always exceeded 95% and, since recovery values for the various experiments were very satisfactory, it was felt that no corrections needed to be made for any small losses incurred in cleanup.

CONCLUSIONS

The selection of acetone as the solvent of choice, since it gave the best recovery values and was essentially trouble-free, was fortuitous since it also resulted in less coextractives. This was determined visually by the deeper yellow color of the extracts when using other solvents, especially benzene-methanol. This has been reported also by Chiba and Morley (1968).

Although the Polytron extraction method was consistently the best method, the differences in recovery over the ultrasonic cleaner and Soxhlet methods were often small. It must be remembered that this method employs only a 30-sec extraction, whereas the other ultrasound and the Soxhlet methods

used 20-min and 8-hr extraction times, respectively. The fact that extraction can be done rapidly means that a large number of samples can be processed in a short time period. The only limiting factor in the number of samples processed would be those imposed by limitations in associated glassware and equipment needed during cleanup. The need for extensive cleanup could be diminished or eliminated by using smaller soil samples. One shortcoming with the Polytron is that, since the generator is made of stainless steel, extraction of soils high in sand wears down the cutting edges of the generator rotor, necessitating rotor replacement after 150-200 extractions. Current replacement cost is about \$35.00.

This method has been used in our laboratory since 1968. On numerous occasions, after Polytron extraction, the soil filter cake was reextracted with the Soxhlet for 8 hr and the extract found to contain negligible amounts of residues, always less than 1% of that amount added to treated soils. It is evident that the deviation between duplicated samples was consistently quite low. Soxhlet extraction is used widely for soil extraction, but it was evident that recoveries varied from one experiment to another, as did the deviation, both possibly due to a channeling effect.

As pointed out by Chiba (1969), a knowledge of the efficiency and of the related factors involved in extraction of residues from soil is still very limited and unsatisfactory. He states that the selection of solvents, the moisture content of the soil, and the soil type are the three key factors which affect the extraction of organochlorine insecticides from soil. It has been attempted here to establish an ideal procedure for obtaining maximum extraction efficiencies using an array of soil types and under a wide variety of conditions. It is hoped that this method may find wide application in soil studies where, in the past, many methods and solvents have been used, making comparisons difficult if not impossible.

In summary it is felt that by using the Polytron, organochlorine insecticide residues can be extracted much faster, more reliably, and more quantitatively from soils than by conventional extraction methods.

LITERATURE CITED

- Babikov, I. O., "Ultrasound and Its Industrial Applications," Consultants Bureau, New York, 1960, 224 pp.
Brinkmann Instruments Inc., Westbury, New York, Bulletin BR 155-B, 1969.
Brown, B., Goodman, J. E., "High Intensity Ultrasonics," Van Nostrand, New York, N.Y., 1965, p 196-220.
Burchfield, H. P., Johnson, D. E., Storrs, E. E., "Guide to the Analysis of Pesticide Residues," Vol. 1, Sect. II A2, Public Health Service, Office of Pesticides, Washington, 1965.
Chiba, M., *Residue Rev.* 30, 63 (1969).
Chiba, M., Morley, H. V., *J. Agr. Food Chem.* 16, 916 (1968).
Duffy, J. R., Wong, N., *J. Agr. Food Chem.* 15, 451 (1967).
Fahey, J. E., Butcher, J. W., Murphy, R. T., *J. Econ. Entomol.* 58, 1026 (1965).
Frye, W. G., *Advan. Biol. Med. Phys.* 4, 282 (1958).
Harris, C. R., *J. Econ. Entomol.* 59, 1221 (1966).
Johnsen, R. E., Starr, R. I., *J. Econ. Entomol.* 60, 1679 (1967).
Johnsen, R. E., Starr, R. I., *J. Econ. Entomol.* 63, 165 (1970).
Mumma, R. O., Wheeler, W. B., Frear, D. E. H., Hamilton, R. H., *Science* 152, 530 (1966).
Saha, J. G., *Bull. Environ. Contam. Toxicol.* 3, 26 (1968).
Schroder, G. W., *Food Eng.* 25, 48 (1953).
Seal, W. L., Dawsey, L. H., Calvin, G. E., *Pestic. Monit. J.* 1, 22 (1967).
Stevens, L. J., Collier, C. W., Woodham, D. W., *Pestic. Monit. J.* 4, 125 (1970).
Trautman, W. L., Chesters, G., Pionke, H. B., *Pestic. Monit. J.* 2, 93 (1968).

Received for review April 12, 1971. Accepted June 17, 1971. Published with the approval of the Director of the Colorado Agricultural Experiment Station as Scientific Series Paper No. 1631. Contribution from the Colorado Agricultural Experiment Station. Supported in part by Western Regional Research Project W-45.