Apparent Increase of DDTR Residues in Wafered Hay[1]

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In a previous study, Ware et al. (1) observed that DDTR residues in alfalfa cubes > field hay > lab-dried hay > green alfalfa, all collected at the same time from the same fields, expressed either as wet or dry weight. Theoretically all values within a sampling should have been the same. The authors suggested that the discrepancies appeared to be related to (a) moisture content of the product at extraction, (b) rate of drying from green to dried form, and (c) cellular disruption in the cubing process making the cell contents more accessible to the solvent.

The following is a detailed laboratory approach to the problem with the sole objective of determining whether DDTR residues are in fact measurably higher in compressed than in noncompressed alfalfa.

Materials and Methods

A Phoenix-grown, 3-months old, standard, 3-wire bale of alfalfa hay was sampled for inherent DDTR residues by multiple coring followed by the usual powdering on a Wiley mill, to pass a 30-mesh screen. The samples (10 g) were extracted 10 minutes in an omnimixer with 300 ml of chloroform-methanol (2:1). After washing to remove the methanol, and drying over sodium sulfate, a 20 ml aliquot of the extract was eluted from a prewashed 4-inch Florisil column with 200 ml of 6% ethyl ether in hexane. All extracts were reduced to 30 ml and dehydrochlorinated with 30 ml of saturated ethanolic sodium hydroxide for a 3-hr period. The extracts were washed, dried over sodium sulfate and reduced to 10 ml held in 12 ml glass stoppered centrifuge tubes.

Samples were analyzed for o,p-DDE and p,p'-DDE using ECGC. Separation was achieved on an 8', 4 mm ID Pyrex glass column, packed with 100-120 mesh chromosorb-W treated with 4% SE-30 and 6% QF-1. Preceding the packing was a 1" plug of anhydrous cadmium chloride

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[1] Contribution to Regional Project W-45, "Residues of Selected Pesticides--Their Nature, Distribution, and Persistence in Plants, Animals and the Physical Environment." University of Arizona Agricultural Experiment Station journal series #1972.

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and a 1" plug of sodium carbonate. Nitrogen gas flow was 65 cc/min. Column, inlet and detector temperatures were 210°C, 225°C and 220°C, respectively.

The experimental hay bale was then transported to the University of California, Davis, Department of Agricultural Engineering for experimental wafering.

Prior to wafering, one-third of the bale was removed and random sections shredded on a hammer mill equipped with a 3/8-inch screen, then mixed in a tumble mill for 5 minutes. This was followed by weighing out 25-27 g subsamples which were compressed according to the schedule of Table 1.

TABLE 1

EXPERIMENTAL WAFERING DESIGN OF ALFALFA HAY FOR DDTR ANALYSES

Cubing Pressure (psi)	100°F	175°F + H_2O		250°F
2,000	8*	8	-	8
4,000	-	8	-	-
6,000	8	8	8	8
8,000	-	8	8	-
10,000	8	8	8	8
12,000	-	8	8	-
14,000	8	8	-	8

* 5 wafers used for DDTR residue analysis and 3 for total protein.

The wafers were formed in a single-die, hydraulically-operated laboratory press as described by Waelti and Dobie (2). Pressure, die temperature and duration of compression time could be pre-set independently, with ranges from 0-14,000 psi, ambient to 300°F., and 3 to 30 seconds, respectively. The hay was forced downward through a cylindrical, 1.25 inch diameter, vertical die by a piston to a movable stop. The stop was forced downward by a predetermined pressure from the piston. This method of compression closely resembles the pressure in a commercial field cuber which forces hay through a die against back pressure generated by die constriction.

Moisture content of the shredded hay was determined to be 7.3%, by heating in an oven for 24 hours at 150°C. In order to simulate field conditions wafers were adjusted to 15% moisture, according to the schedule of Table 1, by adding 2.4 g of water to each 26 g hay sample immediately before compression.

In preparing the wafers, all samples to be compressed at 175°F were processed in a series, as were those at 100°F and 250°F. The press was set to compress all cubes in seven seconds, except at 14,000 psi, which required from 9 to 18 seconds. Eight wafers were made at each parameter. Three were analyzed for protein content at Davis with the remaining five analyzed for DDTR residues at the University of Arizona.

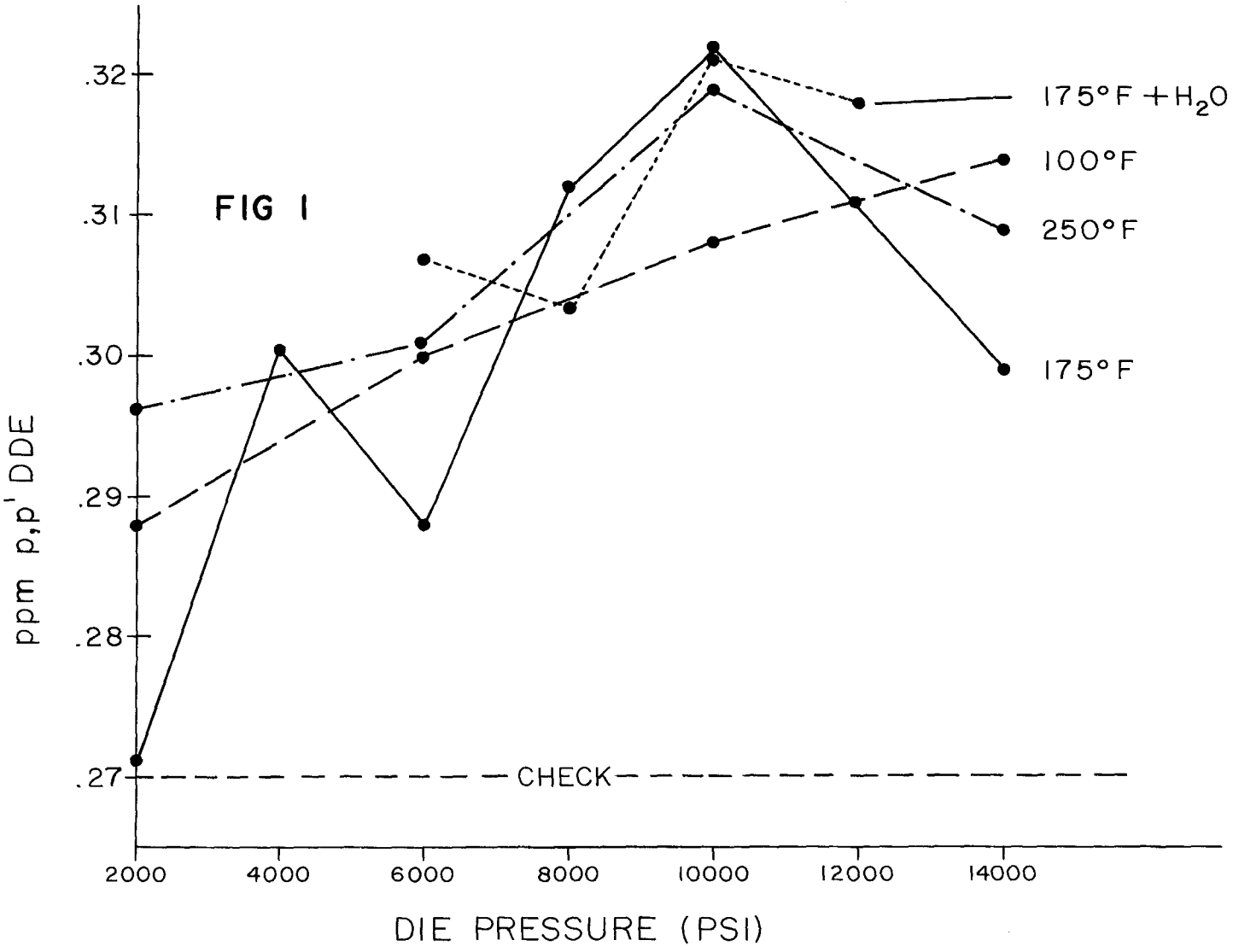
FIG 1
.32
.31
.30
.29
.28
.27
ppm p,p' DDE
175°F + H_2O
100°F
250°F
175°F
CHECK
2000
4000
6000
8000
10000
12000
14000
DIE PRESSURE (PSI)

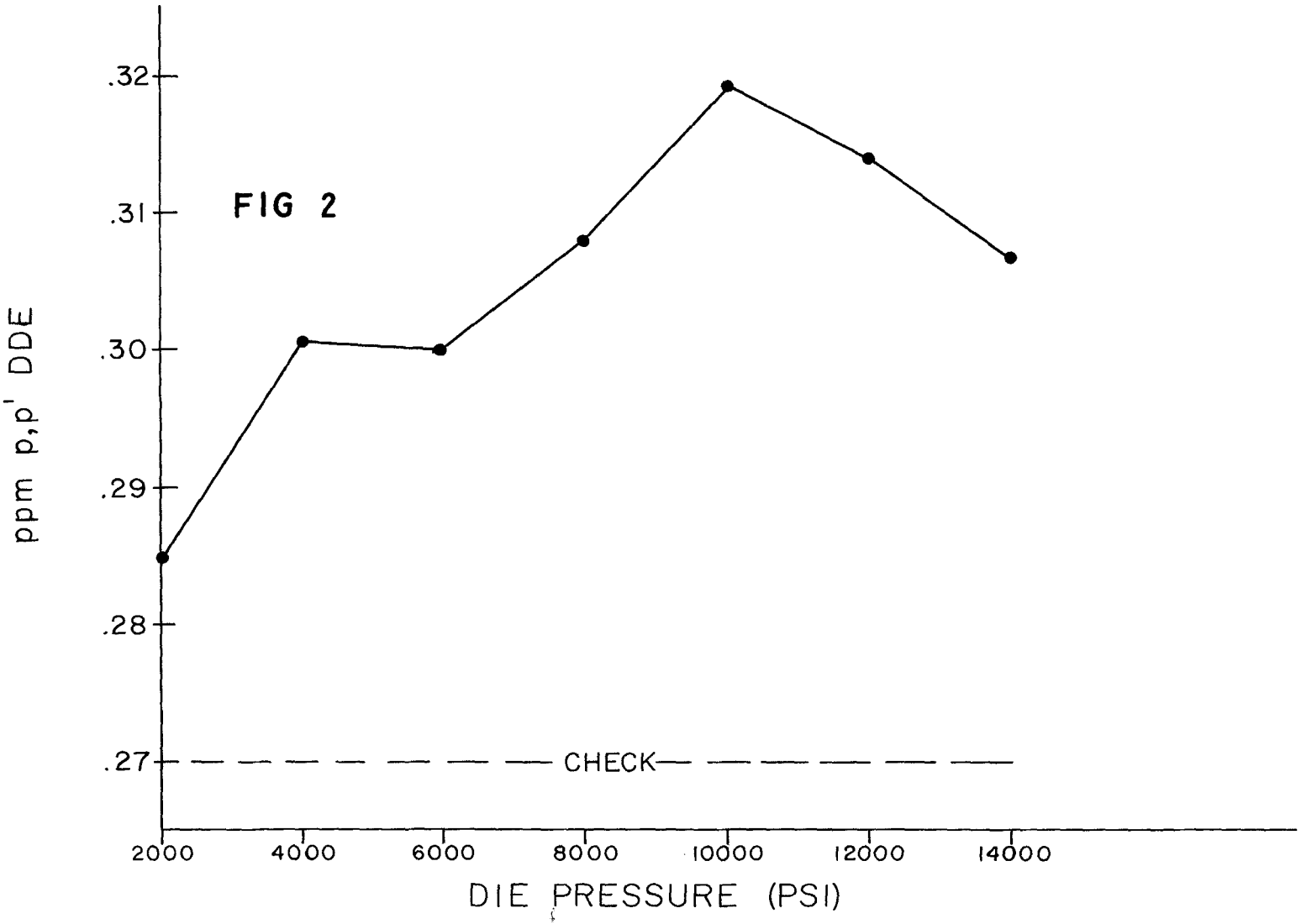
FIG 2
.32
.31
.30
.29
.28
.27
ppm p,p' DDE 126
CHECK
2000
4000
6000
8000
10000
12000
14000
DIE PRESSURE (PSI)

TABLE 2

MEAN PPM O,P- AND P,P'-DDE RESIDUE VALUES FOR 5 REPLICATES OF HAY WAFERED AT DIFFERENT TEMPERATURES AND PRESSURES

PSI	100°F		175°F		175°F + H_2O		250°F	
	o,p-DDE	p,p'-DDE	o,p-DDE	p,p'-DDE	o,p-DDE	p,p'-DDE	o,p-DDE	p,p'-DDE
2,000	.030	.288[a]	.029	.271[a]	---	---	.038	.296[a]
4,000	---	---	.031	.302[ab]	---	---	---	---
6,000	.035	.300[a]	.031	.289[ab]	.037	.307[a]	.032	.302[a]
8,000	---	---	.035	.313[b]	.032	.303[a]	---	---
10,000	.034	.309[a]	.031	.324[b]	.032	.321[a]	.031	.319[a]
12,000	---	---	.031	.311[b]	.035	.318[a]	---	---
14,000	.039	.314[a]	.034	.299[ab]	---	---	.034	.309[a]
Check (0 psi)	.027	.270[a]	.025	.261[a]	---	---	---	---

[a] Within a temperature column, p,p'-DDE means with the same letter are not significantly different at the 0.05 level by Student-Newman-Keuls' test.

After cooling, the wafers were placed in marked plastic bags, sealed, and refrigerated until grinding and analysis.

Results

Table 2 shows the mean ppm values for the 5 replicate groups for both o,p- and p,p'-DDE. Although both o,p- and p,p'- DDE peaks were measured, the error associated with the much smaller o,p peak was greater. Therefore, the effects due to pressure are based on p,p'-DDE analyses.

Figure 1 is a linear plot of wafering pressure versus ppm p,p'-DDE residues observed. A trend is indicated showing that residues increased with increasing pressure to 10,000 psi, then declined. Figure 2 shows a plot of pooled data including all p,p'-DDE residues at a given pressure. The slope trend is clearly evident, peaking at 10,000, and declining through 12,000 and 14,000 psi.

The individual p,p'-DDE residue and protein analyses for each pressure and temperature were subjected to a Student-Newman-Keuls' mean discrimination statistical analysis, which is a form of mean comparison.

Regarding residues, there were no differences between pressures at 100°F, 250°F, and 175°F with water. At 175°F, there were differences between 2,000 psi and 8,000, 10,000 and 12,000, but not 14,000 psi, at the 0.05 significance level.

The mean protein contents in the different pressure groups were different only at 175°F where the 2,000 and 8,000 psi were significantly different at the 0.05 level, with values of 16.0% and 14.5% respectively. Because protein content should have been identical throughout, this reflects on the sampling procedure, indicating variations in stem and leaf proportions of individual samples.

The data support the hypothesis that an increase in wafering pressure is correlated with an increase in evident DDTR residue. This holds true up to 10,000 psi, beyond which the residues decline. There is no evidence of an effect due to die temperature or moisture content. These results, supported by those of the original study, (Ware et al.) produce an analytical phenomenon, for which no simple explanation is available. It does however appear to be a physical relationship between the insecticide molecule and plant cells, altered by pressure and most probably internal or frictional heat. Also indicated is the failure to remove all tissue-bound DDTR residues from dry material when extracted with the most exhaustive solvent systems.

References

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2. WAELTI, HENRY and DOBIE, J. B. ASAE paper 71-115, Amer. Soc. Agric. Eng., St. Joseph, Mich. 49085 (1971).