JOURNAL OF PLANT PROTECTION RESEARCH
Vol. 44, No. 2 (2004)

FAST IDENTIFICATION OF TRACE-LEVEL PESTICIDE RESIDUES IN AGRICULTURAL CROPS APPLYING LOW-PRESSURE GAS CHROMATOGRAPHY/MASS SPECTROMETRY

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Accepted: May 7, 2004

Abstract: A method for the fast identification of trace levels of pesticide residues in agricultural crops was developed using low pressure gas chromatography/mass spectrometry (LP-GC/MS). The final chromatographic determination took 12 minutes per sample while conventional GC/MS required at least 30 minutes. Also, improved peak shapes for dichlorvos, dimethoate, chlorothalonil, pirymethanil, pirimicarb, carbaryl, myclobutanil, flusilazole tebuconazole, fenarimol and iprodione were obtained which generally enabled lower limits of detection. The method was successfully applied to analysis of more than 40 pesticides in 120 samples of fruits, vegetables and cereals. With the aid of LP-GC/MS the number of samples analysed on the particular instrument could be at least doubled.

Key words: pesticide residue analysis, fast chromatography, GC/MS, LP-GC/MS

INTRODUCTION

Pesticides play an essential role in modern agriculture. They are used by farmers to control various pests including weeds, insects, fungi, and microorganisms. They are used to prevent damages to the crops, thereby increasing crop yields and maintaining product quality. However, pesticides may be hazardous for the environment and food consumers' health if used improperly or too frequently. Therefore, many countries have established legal directives to control pesticides in food through maximum residue levels (MRLs) which has led to the development of many methods for monitoring these compounds in a variety of food commodities.

Faster analysis has always been a focus of research investigations of concerned analysts in order to improve the laboratory productivity and reduce costs. The advances made in the past decade led to exciting possibilities in achieving fast gas chromatography (GC) and particularly gas chromatography/mass spectrometry

(GC/MS). Review articles on fast GC have been published recently (Korytár et al. 2002; Matisová and Dömötörová 2003; Maštovská and Lehotay 2003). Low-pressure gas chromatography/mass spectrometry (LP-GC/MS) is one of the options available. This technique makes use of a relatively short (10 m) column of 0.53 mm internal diameter which is operated under reduced pressure using the vacuum required by the mass spectrometric detector. The analytical column is connected to a restriction capillary (0.1–0.25 mm of appropriate length) at the inlet end to provide above atmospheric inlet pressures and allow normal sample injection methods (de Zeeuw et al. 2000).

Some efforts have already been made to adopt low-pressure gas chromatography in pesticide residues analysis. Maštovská et al. (2001) optimised LP-GC/MS conditions for the analysis of 20 pesticides in carrots and the researchers from the University of Almeria (Spain) described some applications of LP-GC in conjunction with tandem mass spectrometry (González-Rodríguez et al 2002; Arrebola et al. 2003; Martínez Vidal et al. 2003).

The objective of this study was to develop a method for the fast identification of 46 compounds including commonly used pesticides, their isomers and metabolites in various agricultural crops using selected ion monitoring (SIM) and full scan determinations. To knowledge of this author there has been no previous work exploring an LP-GC/MS method for a routine qualitative confirmation of results obtained by gas chromatography with selective detectors.

MATERIAL AND METHODS

The samples of fruit and vegetables were extracted with acetone, then partitioned into dichloromethane according to the principle of the Polish Norm PN-EN 12393-2:2000. Crude extracts were subjected to clean-up on silica (Si) disposable SPE columns according to the J.T. Baker application note AN 405. The final extract at a sample concentration of 1 g/ml was ready for the gas chromatography determination.

LP-GC/MS experiments were performed using a Hewlett Packard model 5890 Series II gas chromatograph interfaced to a 5971A mass selective detector operating in electron impact (EI) ionisation mode. Compounds were separated on a DB-5 10 m × 0.53 mm id, 0.88 μm column (Agilent Technologies) connected to a DB-5MS 3 m × 0.2 mm id, 0.5 μm (Agilent Technologies) restriction column at the inlet end with a universal glass press-tight connector (Restek). Carrier gas was helium at 6 psi (constant pressure). The column was held at 80°C for 1 min after injection then programmed at 25°C/min to 280°C which was held for 3 min. Inlet temperature was 250°C. Aliquots of sample extracts 1 or 2 μl were injected manually (splitless).

For comparison purposes conventional GC/MS analysis was performed on the same instrument using a DB-5MS 30 m × 0.25 mm id, 0.25 mm column (Agilent Technologies). The column was held at 80°C for 1 min after injection then programmed at 10°C/min to 280°C which was held for 9 min. Carrier gas was helium at 15 psi (constant pressure). Inlet temperature was 250°C. Aliquots of sample extracts 1 or 2 μl were injected manually (splitless).

RESULTS AND DISCUSSION

In practice, two main approaches can be considered to speed up gas chromatographic separation: (1) the use of small diameter micro-bore capillary columns, and (2) the use of low-pressure gas chromatography (LP-GC). However, in the case of pesticide residue analysis the micro-bore column approach is rather impractical due to unfavourable limits of detection and quick deterioration of column performance with injections of complex extracts (Amirav 1998). Low pressure gas chromatography seems to be a more attractive alternative because wide-bore columns have high sample capacities, thus LP-GC avoids the drawbacks of micro-bore columns. Although somewhat reduced separation efficiency of LP-GC columns was reported it could be compensated for by the selectivity of the detection device used (de Zeeuw et al. 2000). For the above reasons LP-GC approach is preferred in this study.

At the first stage of the experiment, instrumental conditions were optimised to obtain fast separation and good sensitivity. After several trials, it was found that the initial temperature must not be higher than 80°C to allow determination of the earliest eluting compound dichlorvos and the final temperature must not be lower than 280°C to avoid late elution of azoxystrobin. The working range for the carrier gas pressure was found to be 2–8 psi (14–55 kPa); eventually 6 psi was set due to slightly better separation of compounds appearing in the middle section of the chromatogram. In general, compounds eluted from the column at lower temperatures than they did if conventional columns were used. This enabled fast temperature programming which resulted in 2–3 times faster analysis. In this approach, the final chromatographic determination took 12 minutes per sample while it usually required at least 30 minutes using a conventional GC/MS technique.

Subsequently, real samples containing incurred pesticide residues resulting from applications of plant protection products were analysed to evaluate the feasibility of LP-GC/MS approach for the use in routine analyses. The samples were previously analysed by gas chromatography with selective detectors (GC/NPD or GC/ECD) then only those suspected of containing pesticides were reanalysed using LP-GC/MS. The method was successfully used for confirmatory purposes for over 4 mounts and a total of 120 samples of fruits, vegetables and cereals were analysed. LP-GC/MS data for selected ion monitoring analysis of 46 compounds (pesticides, their isomers and metabolites) are given in table 1. The limits of detection listed in

Table 1. Scope of LP-GC/MS analyses

Compound	Ions m/z	Rt min	LOD mg/kg	Commodity
dichlorvos	185, 109	2.80	0.008	cucumbers
trifluralin	264, 306	5.08	0.004	carrots
dimethoate	93, 87, 125	5.29	0.014	apples, cucumbers
quintozene	237, 249, 295	5.51	0.010	apples
pirymetanil	198, 199	5.59	0.002	apples, blackcurrants, cucumbers, strawberries, tomatoes
diazinon	304, 199, 227	5.63	0.007	apples, blackcurrants, carrots, mushrooms

Compound	Ions m/z	Rt min	LOD mg/kg	Commodity
chlorothalonil	266, 264, 268	5.72	0.006	cabbage, cucumbers, tomatoes
pirimicarb	166, 167, 238	5.85	0.007	apples, blackcurrants, plums
vinclozolin	198, 212, 285	6.03	0.005	strawberries
carbaryl	144, 115, 116	6.06	0.010	cucumbers, tomatoes
prometryn	226, 184	6.12	0.005	carrots
metalaxyl	206, 249, 234	6.13	0.009	cucumbers, tomatoes
fenitrothion	260, 277	6.25	0.006	blackcurrants, gooseberries, redcurrants
pirimiphos methyl	305. 290. 276	6.27	0.003	cucumbers, rye, tomatoes, wheat
malathion	127, 173, 158	6.34	0.007	carrots, tomatoes
chlorpyrifos	314, 199, 197	6.43	0.007	carrots, blackcurrants, cucumbers, tomatoes
pendimethalin	252	6.69	0.004	carrots
tolyfluanid	238, 240	6.73	0.005	apples, cucumbers, raspberries, tomatoes, strawberries
captan	79, 149, 80	6.75	0.128	apples, plums
folpet	260, 262	6.80	0.010	strawberries
procymidone	285, 283	6.84	0.004	blackcurrants, cucumbers, raspberries, tomatoes, strawberries
endosulfan α	339, 277, 279	6.99	0.032	blackcurrants, mushrooms, strawberries
DDE pp'	246, 176, 318	7.19	0.003	carrots
dieldrin	237, 261, 277	7.20	0.015	carrots
myclobutanil	179, 150, 181	7.23	0.018	cucumbers
flusilazole	234, 233, 206	7.26	0.006	cucumbers, gooseberries, blackcurrants
bupirimate	166, 208, 316	7.28	0.020	gooseberries
endosulfan β	339, 277, 279	7.44	0.049	blackcurrants, mushrooms, strawberries
DDD pp'	165, 235, 237	7.51	0.003	carrots
oxadixyl	105, 120, 163	7.52	0.010	cucumbers, tomatoes
DDT op'	165, 235, 237	7.53	0.005	carrots
trifloxystrobin	116, 186, 222	7.79	0.008	apples
endosulfan SO_4	385, 387	7.78	0.034	blackcurrants, mushrooms
DDT pp'	165, 235, 237	7.80	0.005	carrots
tebuconazole	250, 252	7.90	0.017	plums
iprodione	314, 316	8.10	0.007	apples, blackcurrants, lettuce, strawberries
bifenthrin	181, 182, 166	8.20	0.005	cucumbers, strawberries, tomatoes
fenpropathrin	265, 181, 209	8.25	0.021	cucumbers
tetradifon	229, 356, 358	8.39	0.015	lettuce
phosalone	182, 184, 367	8.46	0.018	apples, blackcurrants, strawberries
cyhalothrin γ	181, 197, 199	8.61	0.008	blackcurrants, cherries
fenarimol	139, 219, 251	8.67	0.013	apples, gooseberries
cypermethrin	163, 165, 181	9.29 9.34 9.38	0.020 0.024 0.022	apples, blackcurrants
cypermethrin α	163, 165, 181	9.39	0.005	blackcurrants, cherries
deltamethrin	181, 253, 255	10.12 10.27	0.076 0.067	blackcurrants, mushrooms
azoxystrobin	344, 345, 172	10.49	0.085	cucumbers, tomatoes

Rt – retention time, LOD – limit of detection

the table were calculated by extrapolating the signal to noise ratios (S/N) of the pesticide peaks to determine the concentration at which S/N = 3.

A remarkable advantage of LP-GC/MS in comparison with conventional GC/MS was an improved detectability for pesticides of poorer gas chromatographic behaviour since the peaks were sharper and more symmetrical (Fig. 1). In this study, reduced peak tailing was observed for dichlorvos, dimethoate, chlorothalonil, pirymethanil, pirimicarb, carbaryl, myclobutanil, flusilazole, tebuconazole and fenarimol. Furthermore, only very slight thermal degradation of iprodione occurred (Walorczyk 2003).

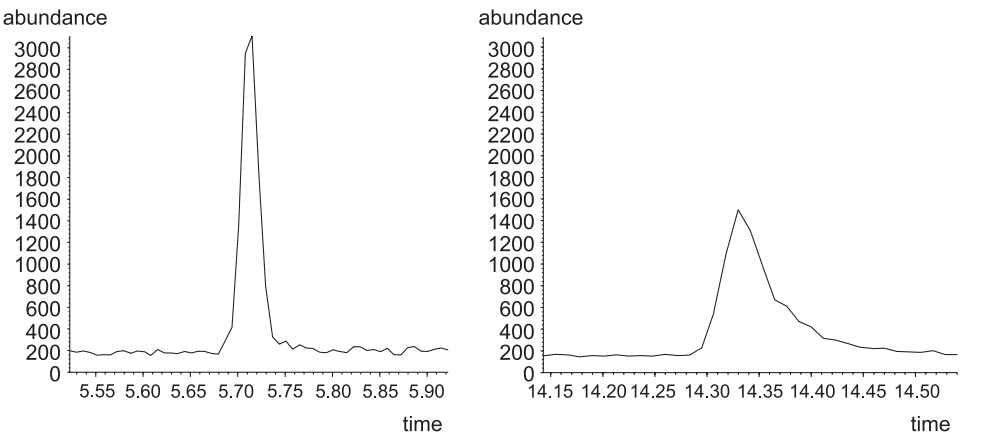

Fig. 1. Peak-shapes of chlorothalonil obtained by LP-GC/MS and GC/MS

The mass spectra produced with LP-GC/MS technique showed direct equivalency with the spectra obtained by the conventional GC/MS. An example of positive identification of tolyfluanid and iprodion in a strawberry sample is given in figure 2. In the same manner also chlorothalonil and pirymiphos methyl in tomatoes, pirymethanil in strawberries, procymidon in strawberries and blackcurrants, iprodione in lettuce, and chloropyrifos, cypemethrin and deltamethrin in blackcurrants were identified in full scan mode.

In conclusion, LP-GC/MS has appeared to be an attractive way of increasing the speed of gas chromatographic analysis because: (1) it can be used with a standard GC/MS instrument, (2) the analysis is considerably faster, (3) a broader range of compounds can be analysed and (4) lower limits of detection for some compounds are possible. However, it must be pointed out that the laboratory sample throughput does not solely depend on the time of the instrumental determination step but also on the time spent on sample preparation (extraction and clean-up). Nevertheless, if the analytical chemist successfully implements faster chromatographic analysis, it is likely that either the number of samples analysed on the particular instrument will be increased or additional projects assigned.

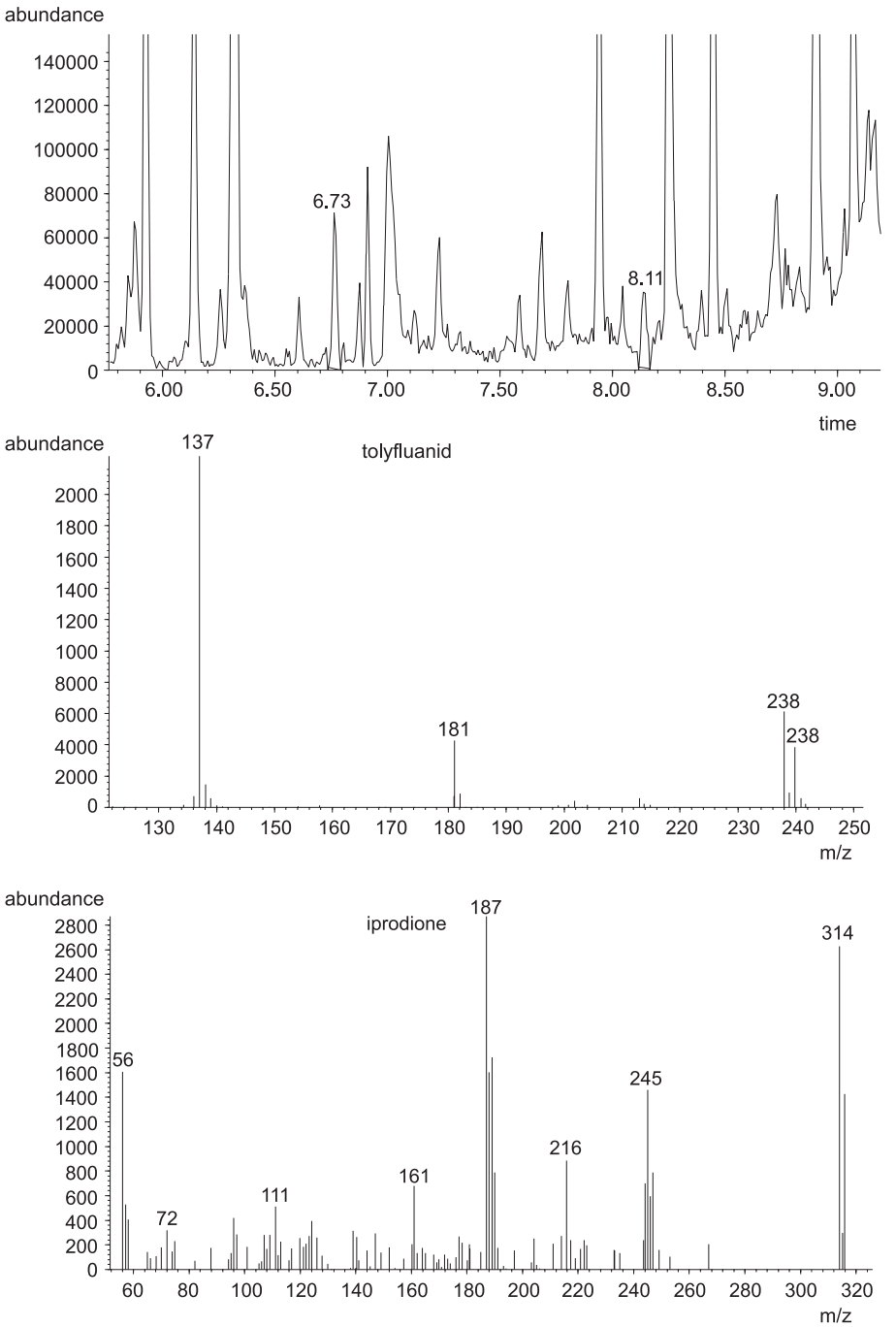

Fig. 2. Mass spectra of tolyfluanid and iprodione present in a strawberry sample

REFERENCES

Amirav A., Tzanani N., Wainhaus S.B., Dagan S. 1998. Megabore versus microbore as the optimal column for fast gas chromatography/mass spectrometry. Eur. J. Mass Spectrom., 4: 7–13.

Arrebola F.J., Martínez Vidal J.L., González-Rodríguez M.J., Garrido-Frenich A., Sánchez Morito N. 2003. Reduction of analysis time in gas chromatography. Application of low-pressure gas chromatography-tandem mass spectrometry to the determination of pesticide residues in vegetables. J. Chromatogr. A, 1005: 131–141.

de Zeeuw J., Peene J., Jansen H.-G., Lou X. 2000. A simple way to speed up separations by GC-MS using short 0.53 mm columns and vacuum outlet conditions. J. High Resolut. Chromatogr., 23 (12): 677–680.

González-Rodríguez M.J., Garrido-Frenich A., Arrebola F.J., Martínez-Vidal J.L. 2002. Evaluation of low-pressure gas chromatography linked to ion-trap tandem mass spectrometry for the fast trace analysis of multiclass pesticide residues. Rapid Commun. Mass Spectrom., 16: 1216–1224.

Korytár P., Janssen H.-G., Matisová E., Brinkman U.A.Th. 2002. Practical fast gas chromatography: methods, instrumentation and applications. Trends Anal. Chem., 21 (9-10): 558–572.

Martínez Vidal J.L., González-Rodríguez M.J., Arrebola F.J., Garrido-Frenich A., Sánchez López F.J., Mora Díez N. 2003. Selective extraction and determination of multiclass pesticide residues in post-harvest French beans by low-pressure gas chromatography/tandem mass spectrometry. J AOAC Int., 86 (4): 856–867.

Maštovská K., Lehotay S.J. 2003. Practical approaches to fast gas chromatography-mass spectrometry. J. Chromatogr. A, 1000 (1-2): 153–180.

Maštovská K., Lehotay S.J., Hajšlová J. 2001. Optimization and evaluation of low-pressure gas chromatography-mass spectrometry for the fast analysis of multiple pesticide residues in a food commodity. J. Chromatogr. A, 926: 291–308.

Matisová E., Dömötörová M. 2003 Fast gas chromatography and its use in trace analysis. J. Chromatogr. A, 1000 (1-2): 199–221.

Walorczyk S. 2003. LP-GC/MS w analizie pozostałości środków ochrony roślin. Prog. Plant Protection/Post. Ochr. Roślin 43 (1): 468–472.

POLISH SUMMARY

SZYBKA IDENTYFIKACJA ŚLADOWYCH POZOSTAŁOŚCI ŚRODKÓW OCHRONY ROŚLIN W PŁODACH ROLNYCH ZA POMOCĄ NISKOCIŚNIENIOWEJ CHROMATOGRAFII GAZOWEJ Z DETEKCJĄ MASOWĄ

Opracowano metodę szybkiej identyfikacji pozostałości środków ochrony roślin w płodach rolnych przy zastosowaniu niskociśnieniowej chromatografii gazowej połączonej ze spektrometrią mas (LP-GC/MS). Końcowe oznaczenie chromatograficzne trwa 12 minut podczas, gdy w konwencjonalnej technice GC/MS co najmniej 30 minut. Ponadto, uzyskano lepsze kształty pików dla dichlorfosu, dimetoatu, chlorotalonilu, pirymetanilu, pirymikarbu, karbarylu, mychlobutanilu, flusilazolu, tebukonazolu, fenarymolu i iprodionu, co przeważnie umożliwiało uzyskanie niższych granic wykrywalności. Opracowaną metodę z powodzeniem wykorzystano w analizie pozostałości ponad 40 środków ochrony roślin w 120 próbkach owoców, warzyw i zbóż. Dzięki zastosowaniu LP-GC/MS możliwe jest co najmniej dwukrotne zwiększenie liczby oznaczeń wykonywanych na danym aparacie.

Book Review

Spaar, D. (Ed.). 2002. Proizvodstvo Grubykh Kormov [Production of Forage Plants and Fodder]. OOO Variant, Torzhok. Vol. 1, 360 pp., Vol. 2, 373 pp. UDK 636.085.53.003, BAK 45.451.89. (In Russian)

This two volume book has resulted from a German-Russian cooperative project titled „Adaptation of agricultural education and increasing the qualifications in Russian Federation". The main sponsors of the project and book were: (1) German Federal Ministry of Consumers Rights, Food Production and Agriculture, and (2) Ministry of Agriculture and Food of the Russian Federation.

Prof. Dieter Spaar – the project leader and the book editor – has invited sixteen specialists from Germany, Russia, and Belarus to contribute to this very interesting and useful treatise. Among authors are: D. Draeger, F. Ellmer, H. Giebelhausen, C. Gienapp, H. Heilmann, F. Hertig, J. Pickert, D. Pieper, A. Postnikov, W. Schlapunov, S. Schumann, W. Schtscherbakov, A Zakharenko.

Volume 1 contains the following chapters:

Chapter 1 "Importance and aims of production of broad grain forage and fodder plants" (p. 7–24).

Chapter 2 "Fodder significance of broad seed plants" (p. 25–33).

Chapter 3 "Technological aspects of harvesting and use of broad seed fodder plants: way and methods of use" (p. 34–170).

Chapter 4 "Production of forage and fodder plants in field conditions" (p 171–185).

Chapter 5 "Technological grounds for growing fodder plants" (p. 186–355) provide information on growing for forage of the following plants: corn (*Zea mays*), crucifers (*Brassica rapa*, *B. napus* ssp. *rapifera*, *B. oleracea*, *Raphanus sativus*, *Sinapis alba*), forage beet cultivars (*Beta vulgaris* var. *rapacea*), forage carrot (*Daucus carota* spp. *sativus*).

Volume 2 contains the continuation of chapter 5 and covers legume family (*Papilionaceae*) including genera – *Trifolium*, *Medicago*, *Melilotus*, *Lotus*, *Galega*, *Onobrychis* (p. 7–47), grasses *Poaceae* (p. 47–87), and mixed crops (p. 88–110).

Chapter 6 "Natural meadows and grasslands" (p. 111–248) deals with variety of grasslands,. Meadows and pastures used as source of green forage and dried hay.

Chapter 7 "Economic evaluation of production of broad seed fodder plants on natural grasslands" (p. 249–264) compares costs of production of forage and hay on grasslands and cultivated fields.

Although the book provides mainly valuable information on establishing, maintaining and harvesting of forage plant and production fodder, in each chapter plant protection topics are broadly covered. Species of weeds, pests and pathogens occurring in each category of plant or crop are listed, characterized and methods of preventing losses and control of pests, diseases and weeds are provided.

In twelve appendices (Nos. 1–12) (p. 265–329) the reader will find many interesting and useful information concerning contents of nutrients in various fodder plants, developmental stages of weed species (BBCH code) and on many other topics.

The chapter "Literature" contains an impressive list of 912 titles of useful publications.

I strongly recommend this book to all persons concerned with growing and protection of fodder and forage plants.

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