JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

The Degradation of the Natural Pyrethrins in Crop Storage

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Prolonged storage of harvested Tasmanian pyrethrum crop from *Tanacetum cinerariaefolium* has resulted in substantial losses of the pyrethrin esters due to the environmental conditions in the storage shed. The generation of heat, the presence of moisture and oxygen, and the microbial activity were identified as possible causes. A pyrethrum crop sample was divided up and stored in different conditions relating to these variables, and the pyrethrins content was monitored over time using a standard method. Temperature was determined to be a critical factor in the rate of the degradation of the natural pyrethrins. Moisture, oxygen, and microbial activity unexpectedly did not play a major role in the degradation. An initial rapid loss of the natural pyrethrins was observed before the pyrethrins content stabilized at a loss of around 65%. This suggests that the plant structure may provide chemical or physical protection to the pyrethrins. In all cases, the majority of the loss was attributed to the pyrethrin I and pyrethrin II esters.

KEYWORDS: Pyrethrum; pyrethrins; *Tanacetum cinerariaefolium*; storage; degradation; temperature; moisture

INTRODUCTION

The natural pyrethrins consist of six insecticidally active esters (see **Figure 1**), which are synthesized by the plant *Tanacetum cinerariaefolium* (pyrethrum). The natural mixture of esters has highly unusual insecticidal properties and has been used for the past 160 years as a botanical insecticide safely and effectively around the world (*1*). Pyrethrum crops are grown in Tasmania for their insecticidal components. Relative to the dry weight of the flower, a pyrethrum daisy contains approximately 1−2% pyrethrins, which are produced throughout the plant, but approximately 94% of the total yield is concentrated in the seeds (*1*).

The insecticide pyrethrum has numerous advantages and disadvantages because of its known instability. It is fast acting and toxic to insects at very low doses, and then, it degrades quickly in the environment making it relatively nontoxic to humans and animals (*2*). However, it is not considered a satisfactory insecticide against agricultural pests due to its instability toward heat, light, and air and the tendency for insects to recover from sublethal doses (*3*). Despite their superior environmental qualities, the general instability of the pyrethrins has considerably restricted their development as all-purpose crop protection agents and has limited their production throughout the world.

The instability of the natural pyrethrins is also causing a storage problem for the Tasmanian pyrethrum company Botanical Resources Australia Pty. Ltd. (BRA). The Tasmanian pyrethrum crop is harvested over a 4−6 week period and is then required to be stored for periods up to 1 year prior to processing. During this storage time, substantial losses of the pyrethrins occur due to the environmental conditions of its storage. The harvested pyrethrum crop typically has a moisture content of about 10%, although moisture contents of up to 20% are occasionally recorded. The crop piles can act like compost heaps and can generate heat-forming "hot spots" throughout the shed that have reached over 100 °C. The substantial losses of the insecticidally active constituents of pyrethrum are a major economic concern for the Tasmanian pyrethrum industry.

From previous studies, it is evident that heat, oxygen, moisture, and microbial action have the potential to play an active part in the degradation of the natural pyrethrins in the crop storage shed (*4*−*21*). The internal plant structure appears to protect the pyrethrins either physically or chemically from degradation (*13*−*21*). Because much of the detail is unknown, further study of the conditions under which pyrethrins degrade during storage seems worthwhile and forms the basis of this present report. This is an investigative and detailed study into the effect that the environment in the crop shed, in particular heat, the presence of oxygen and moisture, and microbial action, has on the degradation of natural pyrethrins in the stored crop over time.

MATERIALS AND METHOD

The experimental method for sampling, preparation, and analysis of pyrethrum samples for pyrethrins content was adapted from an industrial method for pyrethrins analysis (*22*). The method used is described in this section.

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10.1021/jf0304425 CCC: $27.50
Published on Web 12/30/2003

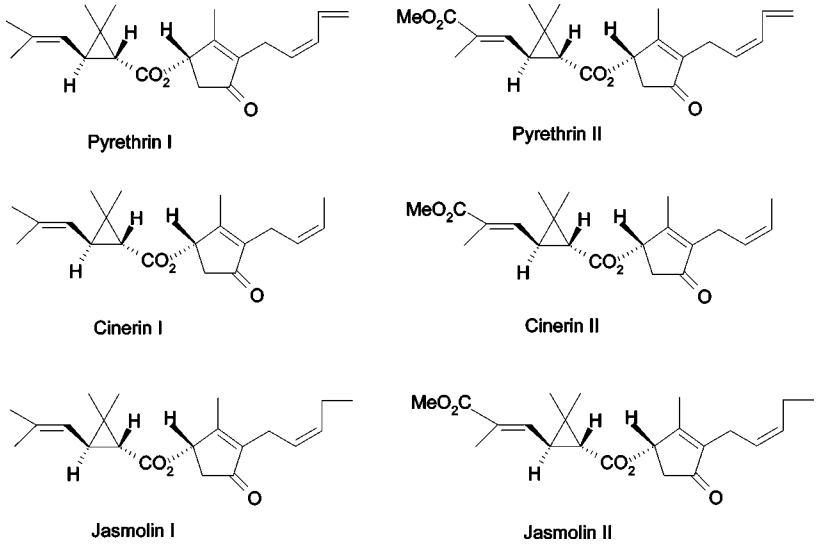

Figure 1. Structures of the natural pyrethrins.

Sampling and Storage Conditions. *Representative Sampling.* A sample of freshly harvested Tasmanian pyrethrum crop, which consists primarily of the flower head and a small portion of the stem, was obtained from the storage shed at BRA. This bulk crop sample was then divided up into small samples for storage under different conditions. An appropriate sampling method known as "coning and quartering" was required to minimize sampling error due to heterogeneity and biological variability in the crop. This method involved heaping the crop into a "cone shape" and then dividing it evenly into quarters. One-quarter was then coned and again divided into quarters. The process was repeated until a sufficiently small sample size (approximately 10 g) was obtained. The small samples were then transferred to 120 mL polystyrene sample containers with polypropylene screw top lids for storage under different environmental conditions relating to moisture, oxygen, and temperature. All samples were kept in the dark during storage.

Moisture Conditions. Three different levels of moisture in the crop were compared. A portion of the crop was freeze-dried using a Javac Breda Scientific LY-5-FM freeze-dryer for 48 h to remove the moisture present in the crop. The freeze-dried crop was then packaged in small containers and sealed. The original moisture (~10%) pyrethrum crop was also packaged in a series of small containers. The remaining third of the samples had the moisture content increased to approximately 20% by adding 1.0 ± 0.1 mL of deionized water to each small sample (10 g) in the polystyrene containers. The moist sample was mixed thoroughly by shaking and then allowed to equilibrate for 24 h.

Atmospheric Conditions. Samples were stored in oxygen deficient (nitrogen), air and oxygen rich (oxygen) atmospheres to test the effect of oxygen on the degradation of the pyrethrins. For storage under nitrogen, uncapped sample containers filled with crop, together with their screw caps, were placed in a glovebag. The glovebag was clamped closed. The gas inlet was connected to a three-way tap that was also connected to a vacuum line and nitrogen inlet. Nitrogen gas (BOC, High Purity) was connected through a drying tower. The glovebag was first evacuated using the vacuum line and then flushed with nitrogen twice before being filled again with nitrogen gas. During the second flushing stage, the samples were gently stirred to remove any air pockets. After the glovebag had been filled with nitrogen for the third time, the sample containers were capped tightly. Upon removal from the glovebag, each container was sealed inside a plastic bag to provide further protection. For storage in an oxygen rich environment, the crop was packaged in the sample containers and flushed with oxygen for 30 s with constant stirring. The containers were quickly capped upon removal of the oxygen inlet.

Temperature Conditions. Ten small samples for each storage condition listed in **Table 1** below were stored in ovens at each of the temperatures 20 ± 2, 40 ± 2, 60 ± 4, 80 ± 5, and 100 ± 8 °C.

Microbial Conditions. The same scheme was adopted for the packaging of samples for sterilization. A large number of small samples, 60 for each condition listed in **Table 1** excluding those packaged under oxygen gas, were sent to the Australian Nuclear Science and Technology Organization (ANSTO) (Lucas Heights, NSW, Australia) for sterilization by γ-radiation. Half of the samples was sterilized at 25 kilograys (kGy), and the other half of the samples was sterilized at a stronger dose of 50 kGy. The samples were held waiting for sterilization at ANSTO for 30 days in cold storage at −80 °C. These samples were in transit between ANSTO (NSW) and the School of Chemistry (Hobart) for 6 days, including through quarantine. Upon return, representative sterilized samples were analyzed for pyrethrins content, and the remainder was put into ovens at the five temperatures as part of the rate study.

Table 1. Storage Conditions

condition	moisture content	atmosphere
1	freeze-dried (0%)	nitrogen
2	freeze-dried (0%)	air
3	freeze-dried (0%)	oxygen
4	normal (10%)	nitrogen
5	normal (10%)	air
6	normal (10%)	oxygen
7	moist (20%)	nitrogen
8	moist (20%)	air
9	moist (20%)	oxygen

Rate Study. Preliminary experiments demonstrated that the rate of degradation significantly increased as the temperature increased. Therefore, samples were periodically removed from the ovens and analyzed at shorter intervals as the temperature increased, e.g., weekly at 20 °C and hourly at 100 °C.

Analytical Method. Hexanes (EM Science, HPLC grade) was the most widely used solvent in the extraction and analysis of the samples. The quality of the solvent was assured by checking the absorbance at 223 nm using high-performance liquid chromatography (HPLC).

Grinding. Samples with a high moisture content were dried in an oven at 50 ± 5 °C for 1 h prior to grinding to achieve optimum performance from the grinder and not degrade the sample. Samples were ground using a Breville Coffee 'n' Spice grinder for 1 min per sample. Preliminary experiments showed that a grinding time of 1 min was optimal for efficiency and efficacy. The moisture content was determined by the weight difference of 0.5 ± 0.001 g of each ground sample after 30 ± 10 min drying at 100 ± 10 °C.

Extraction. Ground pyrethrum crop powder (0.5 ± 0.0001 g) was accurately weighed into a 25 mL volumetric flask and made up to volume by adding 24.5 mL of hexanes (EM Science, HPLC grade), assuming 0.5 g of powder would occupy a volume of approximately 0.5 mL. The flask was placed in an ultrasonic bath for 20 min, inverting and shaking three times in that period. The flasks were stored in dark

Table 2. HPLC Specifications

instrumentation	specification
pump	Millipore Waters 501 HPLC pump
autoinjector	Millipore Waters 717 plus autosampler
column	5 μm silica (8 mm i.d. $\times$ 10 mm internal length) compressed in a Millipore Waters Radial Compression Module
detector	Millipore Waters lambda-max model 481 LC spectrophotometer
data recorder	Profound 486, DOS operating system
software	Maxima 820, chromatography workstation

conditions for at least 4 h (most often overnight) to allow the solids to settle before the extract was filtered through a 0.45 μm Teflon syringe filter to obtain a hexane solution suitable for HPLC analysis.

Cellulose Experiment. Pyrethrum crop (20 g) was ground for 1 min and then extracted with 500 mL of hexanes (EM Science, HPLC grade). The extract was filtered, and the majority of the hexanes was evaporated under reduced pressure until a concentrated pyrethrins solution remained of approximately 10 mL. This was combined with 20 g of a finely ground cellulose powder and thoroughly mixed, and the residual solvent was allowed to evaporate. The pyrethrin−cellulose powder was divided into 10 70 mL polypropylene containers, capped, and placed in the oven at 100 °C. The containers were sampled periodically over 24 h. The pyrethrum−cellulose powder was extracted using the same sample preparation as the ground pyrethrum crop samples explained above.

Reference Solutions. The standard reference solution was a sample of refined pyrethrum extract containing 20% pyrethrins, supplied by BRA with a certificate of analysis stating its concentration by the AOAC 9th edition mercury reduction method of analysis (reference no. 936.05). A reference solution containing 0.4 g/L pyrethrins was prepared by accurately weighing 500 mg of the standard reference solution into a 50 mL volumetric flask and making up to volume with hexane. A 10 mL aliquot of this solution was then quantitatively transferred to another 50 mL volumetric flask and made up to the volume using hexane. This reference solution was used as an external standard for HPLC to determine the pyrethrins content in the unknown samples.

HPLC Analysis. The analysis of the pyrethrins content was performed using normal phase HPLC. A silica column was employed, and an isocratic mobile phase of hexanes and THF was used. The specifications are listed in **Table 2**. The operating conditions employed were based on the standard method for analysis of pyrethrins and are shown in **Table 3**.

The concentration of the six esters in unknown samples was determined by a comparison with the peak areas of the standard reference solution of known concentration. The moisture content of each unknown sample and the weight and dilution of the sample were taken into account when determining the concentration of the pyrethrins. The concentrations of each of the six esters were also determined. A typical chromatogram of pyrethrum crop is shown in **Figure 2**. The results obtained from the experiments were correlated and then statistically analyzed using the software package JMP (version 5.0, SAS Institute Inc., New York) for Windows.

RESULTS AND DISCUSSION

Because of the heterogeneous nature of the pyrethrum crop, the error associated with sampling was expected to be high. The crop consists of primarily the flower head with small variable portions of the stem. A method known as coning and quartering was employed to divide the bulk crop into smaller samples; however, variations in pyrethrins concentration were still observed. By analyzing a group of similar samples, the scatter in pyrethrins content was determined to be 7.37%.

Temperature. The effect of temperature on the degradation of the natural pyrethrins was examined at 20, 40, 60, 80, and 100 °C. Small samples were stored in ovens at the respective temperatures and were sampled periodically depending on the temperature: the higher the temperature, the shorter the sampling

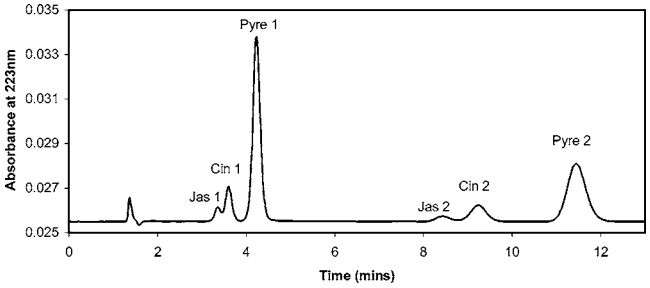

Figure 2. Chromatogram of pyrethrum crop.

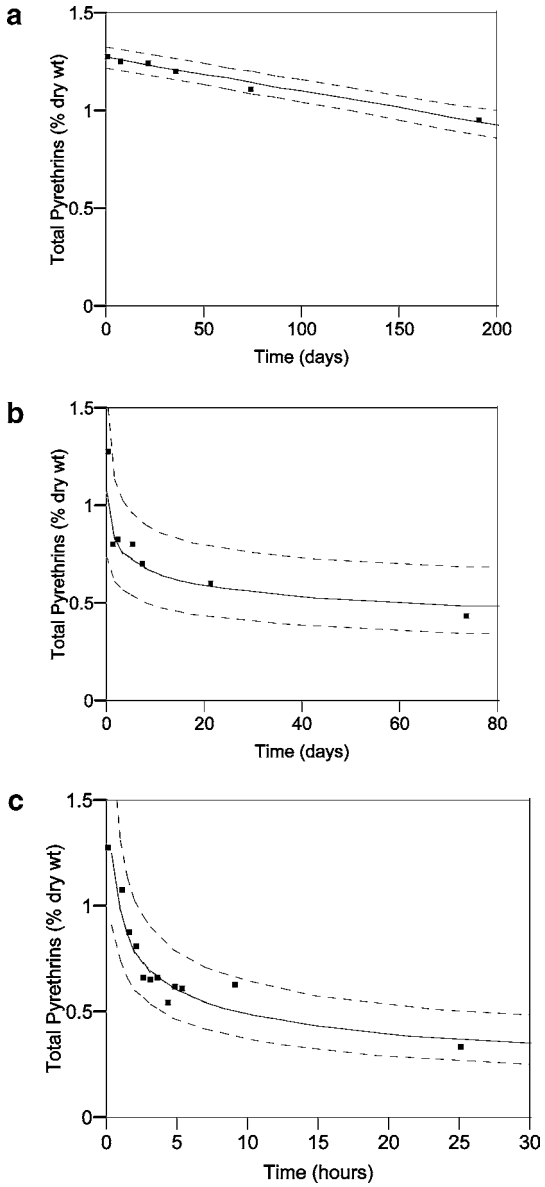

Figure 3. (**a**) Degradation of pyrethrins at 20 °C. (**b**) Degradation of pyrethrins at 60 °C. (**c**) Degradation of pyrethrins at 100 °C.

intervals. The results for the temperature studies at 20, 60, and 100 °C are reported in **Figure 3**.

The *x*-axis represents time in days or hours for 100 °C, while the *y*-axis is the percentage of the natural pyrethrins per dry weight of the crop. All plots contain confidence intervals, which display the upper and lower 95% confidence limits reflected in the variation in the error and in the parameter estimates. In **Figure 3a**, the degradation of the pyrethrins was monitored over 190 days at 20 °C, and during this time, a 26% loss in the natural pyrethrins was observed. At 60 °C, the majority of the

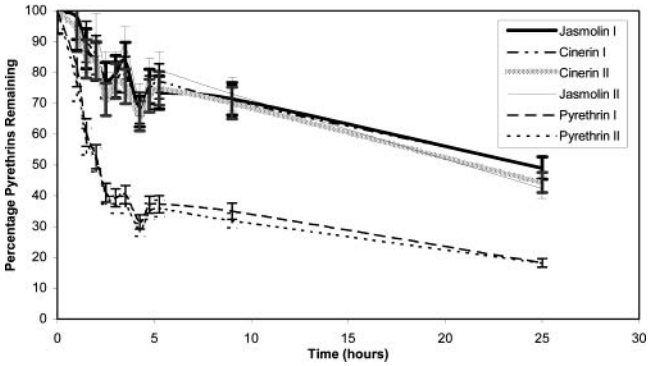

Figure 4. Percentage remaining of individual esters at 100 °C.

Table 3. Operating Conditions for HPLC

mobile phase	95:5 hexanes:THF[a]
stationary phase	5 μm silica
flow rate	2.5–3.5 mL/min
injection volume	5 μL
run time	13 min
wavelength	223 nm

[a] The mobile phase was recovered by distillation and reused.

degradation occurred within the first 5 days, and after 75 days, the total loss was 65%. A more rapid loss in pyrethrins content to the same extent was experienced in samples stored at 100 °C. After 24 h, the loss of pyrethrins was 68%. At this temperature, the pyrethrins degrade rapidly in the first 4 h. However, like the degradation pattern at 60 °C, the degradation stabilizes and does not tend to zero concentration.

The degradation patterns of each of the six pyrethrin esters were also of interest. The esters are present in pyrethrum in the ratios of jasmolin:cinerin:pyrethrin as 1:3:10 (*23*). To directly compare the degradation of each ester, **Figure 4** shows the percentage remaining of the starting concentration for each of the esters.

Figure 4 reveals an interesting trend as the jasmolins and cinerins show a very similar pattern of percentage losses at this temperature. The pyrethrins, however, degrade to a much greater extent over the same time frame. The majority of the decrease in total pyrethrin content seen in **Figure 3c** is attributed to the large percentage loss of Pyre 1 and Pyre 2. The common feature of these two molecules is the conjugation on the side chain. These molecules are known to undergo a thermal isomerization reaction (*12*); however, although the isopyrethrins are very similar in structure to the pyrethrins, they were not detected by HPLC. This suggests that isomerization of the pyrethrins either does not occur or is closelyfollowed by further reactions.

Githinji postulated in 1980 that the thermal degradation of pyrethrins could be explained by the chemical reaction theory (*14*). However, to correctly formulate the law of the rate of reaction, it is essential to know the molecular process by which the resultant reaction is actually brought about. In the absence of this knowledge with respect to the natural pyrethrins (which consist of six complex chemicals), it was found necessary to adopt the method of Noyes and Sherrill, whereby the experimental data are analyzed in order to establish the order of the reaction (*14*). In theory, the order of the reaction n can be determined by assigning different values of n and determining the most linear model from the experimental data. Therefore, n was assigned the values 0, 1, 2, 3, and 4. **Table 4** displays the rate equations for each value of n.

Table 4. Rate Equations

reaction order (n)	equation
0	$[Py]_t = [Py]_0 - kt$
1	$\ln [Py]_t = \ln [Py]_0 - kt$
2	$1/[Py]_t = 1/[Py]_0 + kt$
3	$1/[Py]_t^2 = 1/[Py]_0^2 + 2kt$
4	$1/[Py]_t^3 = 1/[Py]_0^3 + 3kt$

Table 5. R_{adj}^2 Values as a Measure of Linearity

N	20 °C	40 °C	60 °C	80 °C	100 °C
0	0.9781	0.4546	0.3870	0.5143	0.4065
1	0.9879	0.5512	0.6112	0.5620	0.6200
2	0.9940	0.6477	0.8048	0.5972	0.8130
3	0.9966	0.7378	0.9248	0.6161	0.9193
4	0.9959	0.8163	0.9788	0.6177	0.9297

Table 6. Rate Constants

temp (°C)	k ([PY]/day^{-1})
20	0.001546
40	0.006265
60	0.01003
80	0.1708
100	0.9724

By this method, the coefficient of regression was used to determine the linearity of each plot and **Table 5** summarizes the results.

From this table, the order of the reaction is not clear. Each increase in order results in an increase in linearity. This trend indicates that the degradation reaction is a complex mechanism. This may be due to the combination of differential rates of the degradation of the six esters that constitute the natural pyrethrins or it may be due to possible degradation reaction mechanisms such as polymerization and oxidation reactions being superimposed. Alternatively, the complexity of the loss of pyrethrins may be caused by the dependence on the diffusion of the pyrethrins from the seeds.

The reaction is not zero order, because a linear plot does not result when the $[Py]_t$ is plotted against time at temperatures other than 20 °C. The first-order equation introduces the greatest step in linearity. The reaction causing the degradation of the pyrethrins is assumed to be first-order, as suggested by Githinji and supported by the greatest increase in linearity from the R_{adj}^2 values.

The rate constants for each of the five temperatures were determined by plotting the first-order rate equation and are presented in **Table 6**. For most chemical reactions, the rate constant k increases as the temperature increases. This is consistent with the degradation of the natural pyrethrins. The parameters of the Arrhenius expression can be estimated using eq 1.

$$k = Ae^{-\frac{E_a}{RT}}$$

where k = rate constant, A = constant, E_a = activation energy, R = gas constant (8.314J/mol K), and T = absolute temperature. A plot of ln k vs $1/T$ (**Figure 5**) gives a linear model with a slope equal to $-E_a/R$ and an intercept of Ln A.

The relationship between the rate constants and the temperature is significant with an R^2 value of 0.9013. The significance

Table 7. Parameter Estimates for the Arrhenius Equation

parameter	estimates	prob > (*t*)	95% confidence interval
A	8.07×10^9	0.0133	$8604 \leq \beta \leq 7.54 \times 10^{15}$
$-E_a/R$	−8731	0.0087	$-13\,262 \leq \beta \leq -4200$

Table 8. Activation Energies for the Individual Esters

ester	activation energy (kcal/mol)
Jas 1	17 ± 10
Cin 1	17 ± 10
Pyre 1	18 ± 8
Jas 2	18 ± 17
Cin 2	17 ± 11
Pyrethrin II	18 ± 8

of the parameter estimates used for the Arrhenius equation is addressed in **Table 7**.

These parameters can then be substituted into the Arrhenius expression to give

$$k = 8.1 \times 10^9 e^{-8731/T}$$

The activation energy for the degradation of the pyrethrins is 17 ± 9 kcal/mol if the reaction is classified as first-order. Similar studies were performed for second-order kinetics, and the activation energy was determined to be 19 ± 8 kcal/mol. These values are very similar, so the classification of the order of reaction is not important in determining the activation energy.

Similar to the degradation pattern of the total pyrethrins, the classification of the reaction order for each of the six esters was not clear. This indicates that the complex mechanism for the degradation of the total pyrethrins is not due to the combination of differential rates of the six esters. The activation energy for the degradation of each ester was determined using first-order kinetics. **Table 8** summarizes the results. The activation energies are very similar, which suggests that the esters are behaving in a similar manner. Pyre 1 and Pyre 2 degrade at a faster rate, however.

Taking into account the 95% confidence interval, the activation energies for the degradation of the total pyrethrins and each of the esters are consistent with Githinji's value of 14.7 kcal/mol and Shene's value of 12.1 kcal/mol (*5*, *14*). These values are also within close range of the activation energy for the thermal isomerization reaction of 24.6 kcal/mol (*12*).

Moisture and Oxygen. The degradation of the natural pyrethrins was monitored over time when pyrethrum crop samples were stored under conditions relating to moisture and oxygen. **Figure 6** shows the results without the error bars of 7.37% to show the trends more clearly.

The presence of moisture and oxygen does not cause the degradation of the natural pyrethrins. Losses in pyrethrins content are still observed in the absence of water and oxygen. There is evidence in **Figure 6** to suggest that the percentage loss of pyrethrins is greater in freeze-dried material because the three data series with freeze-dried crop have lower concentrations than the other conditions after 8 h. The increased losses may be an artifact of the freeze-drying process, e.g., cell disruption. Alternatively, the chemical reactions causing the degradation may be inhibited by the presence of water and this is supported by the greater loss in Pyre 1 and Pyre 2 concentrations and not the other esters. If a greater quantity of the esters were exposed by the freeze-drying process, then a consistent degradation would be expected for all esters, not just the Pyre 1 and 2 esters.

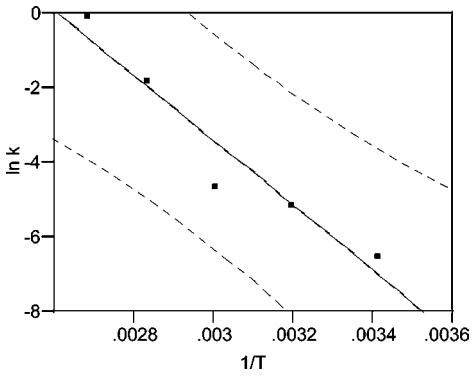

Figure 5. Arrhenius plot.

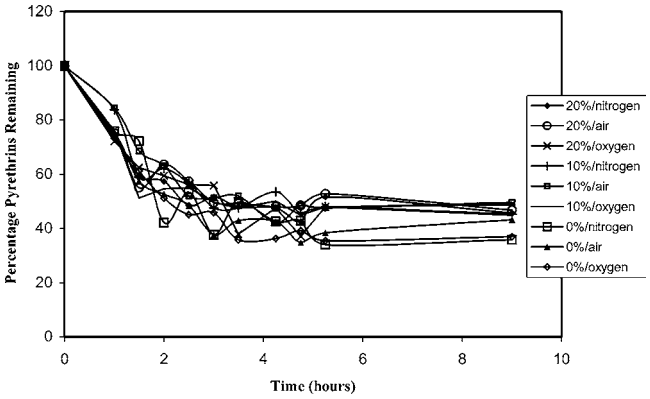

Figure 6. Degradation of pyrethrum crop at 100 °C. The effect of moisture and oxygen.

Microbial Degradation. Microbes may cause the pyrethrins to degrade in two different ways: indirectly through the composting activities generating heat in the shed or directly through metabolizing the pyrethrins. The experiments on microbial activity were completed to test the second theory that the microbes are directly involved in the degradation process. To test this, it was necessary to sterilize the crop samples to kill any microbes present on the plant surface. γ-Radiation was employed for sterilization of the pyrethrum crop samples.

The samples were sent to ANSTO for sterilization by γ-radiation. Two levels of radiation were employed, 25 and 50 kGy. The standard dose required for sterilization of microbes is 25kGy. A stronger radiation dose (50 kGy) was used to destroy soil microbes that were likely to be present in the pyrethrum crop samples. **Table 9** summarizes the percentage losses of the pyrethrins during the sterilization procedure.

Taking into account sampling and analytical errors, some interesting trends were found in in the first section of **Table 9**. The second section of **Table 9** similarly summarizes the results for the samples sterilized at 25 kGy. The negative values are due to the errors associated with the determination of the presterilization and poststerilization values.

The two levels of radiation degraded the pyrethrins in the samples to different extents. At the lower dose, the average total loss is 22%, whereas at 50kGy the average is 42.5% loss in the total pyrethrins. The percentage losses of each of the esters are also dependent upon the radiation doses. From examining the percentage losses of each ester, it is evident for all treatments that the majority of the losses can be attributed to the Pyre 1 and Pyre 2 esters. At the 50 kGy radiation level, over 50% losses in these esters have resulted. The approximate stability of each

Table 9. Percentage Losses in Pyrethrins Content during Sterilization at 50 and 25 kGy

%	total	Jas 1	Cin 1	Pyre 1	Jas 2	Cin 2	Pyre 2
			Losses at 50 kGY				
0%/air	60.0	18.7	5.5	81.6	14.4	22.5	82.4
0%/N_2	60.9	20.8	7.0	82.6	15.5	22.5	82.9
10%/air	36.6	5.9	2.2	48.2	8.8	9.0	55.9
10%/N_2	41.3	18.3	9.0	52.2	18.6	17.8	56.5
20%/air	28.8	22.9	14.8	31.2	14.0	20.9	36.2
20%/N_2	27.2	18.8	11.1	30.2	11.9	18.7	34.7
average	42.5	17.5	8.3	54.3	13.9	18.6	58.1
			Losses at 25 kGy				
0%/air	38.6	18.7	7.4	50.1	9.2	18.3	53.0
0%/N_2	31.2	1.8	−6.5	45.6	−3.5	7.4	49.2
10%/air	18.9	14.1	0.0	25.1	1.0	7.4	28.4
10%/N_2	21.7	7.9	2.9	27.5	3.9	8.7	32.5
20%/air	10.7	6.3	−2.2	10.8	−1.7	8.4	18.0
20%/N_2	11.9	12.5	3.0	12.9	0.3	9.9	15.6
average	22.2	10.2	0.7	28.7	1.5	10.0	32.8

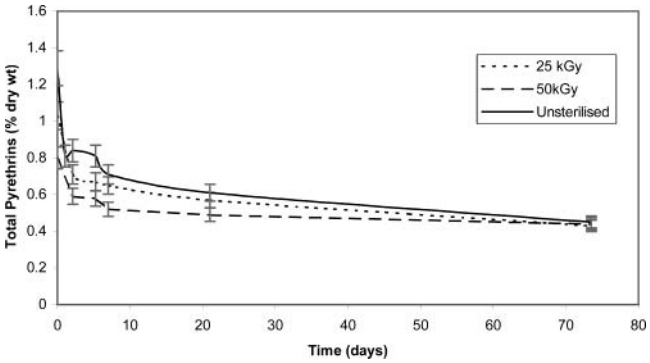

Figure 7. Comparison of sterilized and unsterillized samples at 69 °C.

of the esters is ordered as Cin I > Jas II > Cin II > Jas I > Pyre I > Pyre II.

The moisture content in each sample also points to another trend in the degradation reaction. It appears that as the moisture content decreases, the amount of pyrethrins degradation increases. The freeze-dried samples lost twice the pyrethrins content than the samples stored in 20% moisture. The results from the normal moisture samples (10%) fit proportionally between these two extremes. Looking at the percentage loss in the individual esters for the three moisture levels, again it is the Pyre 1 and 2 esters that have caused the greatest variation. This suggests that the reaction causing degradation of the Pyre 1 and 2 esters is inhibited by the presence of water. This finding adds to the significance of the findings and the possible causing factors described earlier in the moisture and oxygen experiments in unsterilized samples.

Another finding from **Table 9** relates to the conditions under which the samples were stored. There is no significant difference between the degradation patterns of the samples stored in atmospheres of air or nitrogen. This indicates that the reaction causing the decomposition of the esters by γ-radiation does not involve oxygen.

The degraded samples returned from ANSTO were still used to determine the effects of heat, oxygen, and moisture in the absence of microbes on degradation of the natural pyrethrins and to see if these results differed from those when microbes were present. It was assumed that the degraded metabolites did not interfere with the degradation of the remaining pyrethrins. **Figure 7** summarizes the results from the temperature experiment at 60 °C.

The three different conditions relating to sterilization have different degradation patterns, but the series converges to the same level. The starting point for each curve is different; that is, the 50 kGy samples had degraded significantly before being placed in the temperature experiments. Therefore, this initial degradation may cause the latter degradation to be less. At 25 kGy, the same effect is apparent to a lesser extent. Assuming that the microbes were all destroyed, the natural pyrethrins continued to degrade despite the absence of microbes in all of the conditions relating to temperature, oxygen, and moisture content. Therefore, it can be concluded that microbes are not directly involved in the degradation process. However, the microbes may be indirectly involved in generating heat, known to accelerate the reaction, by a composting effect in the crop shed.

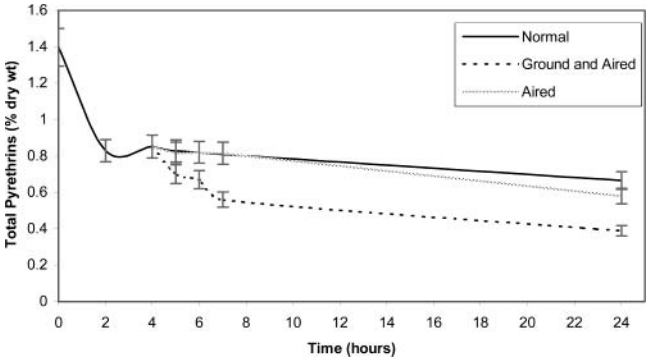

Figure 8. Comparison of ground and aired crop at 100 °C.

Protection of the Pyrethrins by the Plant. It was deemed necessary to investigate why the degradation pattern of the total pyrethrins and each of the esters occurred rapidly and then appeared to stabilize over time. This phenomenon was first recognized by Tattersfield and Martin in the early 1930s (*13*). Tattersfield concluded that the pyrethrins were protected from degradation by the plant due to particle size or to cellular inclusion. However, in 1966, MacIver dismissed this idea and suggested that the stabilization was due to naturally occurring antioxidants in the plant, which prevented the pyrethrins from degrading any further (*21*).

On the basis of the conflicting conclusions of these two studies, it is suggested that the pyrethrins are protected either physically or chemically by the structure of the plant. It is known that the natural pyrethrins are stored in the oil gland on the surface of the seeds. Two possible theories were postulated based on the results of the temperature, moisture, oxygen, and microbial experiments. The first theory proposes that there is a rate-limiting compound that has been used up. Conversely, the second theory suggests that the pyrethrins are protected by the plant structure.

Relating to theory one, it was thought that the oxygen content in the containers might be used up and therefore stop the degradation reaction as the samples were stored in capped, gastight containers. Second, relating to theory two and based on the findings of Tattersfield, it was thought that the pyrethrins might have been bound up in the plant structure allowing protection from environmental conditions (*17*). Also, it is possible that the pyrethrins are stored in other areas in the seed structure apart from on the surface in the oil glands. The action of grinding may expose bound up pyrethrins to the environmental conditions.

To test these two theories, an experiment was conducted involving further grinding and aerating samples when the degradation started to stabilize, i.e., after 4 h at 100 °C. The results from this experiment are represented graphically in **Figure 8**. The samples were placed in an oven at 100 °C and

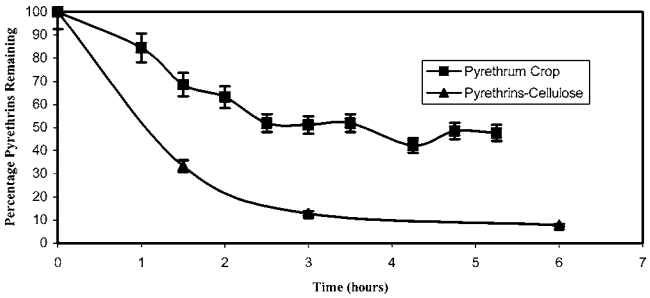

Figure 9. Pyrethrins–cellulose powder degradation at 100 °C.

sampled periodically and then analyzed for pyrethrins content.

As previous results suggested, oxygen content did not make a significant difference on the pyrethrins content. However, grinding the samples and placing them back in the oven at 100 °C caused the pyrethrins to continue to degrade before levels stabilized again at a lower concentration. The samples were ground using the Coffee 'n' Spice grinder, and samples could not be ground very finely. If the sample could be ground fine enough, it is expected that the pyrethrins will completely degrade to a zero pyrethrins concentration.

Cellulose Experiment. In another experiment to support the theory that the pyrethrins were protected by the plant structure, a hexane extract containing the pyrethrins was combined with finely ground cellulose powder. Cellulose was selected because it is an inert, natural plant constituent, which would more closely mimic the pyrethrum plant than other alternatives such as silica gel. It was assumed that the cellulose played no part in the degradation. According to MacIver, the antioxidants, which may provide protection, are not extracted from the plant with hexanes (*21*). This pyrethrum−cellulose powder was divided into small samples and placed in the oven at 100 °C. A sample was removed periodically from the oven and analyzed using HPLC. The results from this experiment are graphed in **Figure 9**.

Figure 9 shows the pyrethrins content in the pyrethrins−cellulose plot decreasing to 94% of the starting value within 6 h, as compared to 65% in the normal pyrethrin crop. Therefore, the pyrethrins degrade completely in the absence of the plant structure, suggesting that some protection is afforded either chemically or physically by the plant structure.

These results are consistent with the conclusions of Tattersfield et al. and MacIver (*13*, *17*, *21*). The grinding experiment seemed to suggest that the stabilization of the pyrethrins content is due to the sample particle size or cellular inclusions present in the plant as suggested by Tattersfield (*13*, *17*). Without further experiments though, this does not discount the convincing findings by MacIver regarding chemical protection in the form of antioxidants (*21*).

CONCLUSION

This project was successful in achieving its aims. Unexpected and useful discoveries were made, which have identified conditions that cause the degradation of the natural pyrethrins in storage, which may be considered when implementing measures to provide better storage conditions for pyrethrum crop.

The most critical environmental condition causing the degradation of the natural pyrethrins was determined to be the temperature at which the crop is stored. Increases in temperature significantly increase the rate of degradation, so the generation of heat in the storage shed should be kept to a minimum. The presence of moisture and oxygen does not cause the degradation of the natural pyrethrins. There is evidence to suggest that the percentage loss of pyrethrins is significantly greater in low moisture material.

Exposure to γ-radiation, through the sterilization treatment, caused the natural pyrethrins to degrade to a significant extent. The degradation again appeared to be inhibited by the presence of moisture, which adds to the significance of the findings in the moisture and oxygen experiments in unsterilized samples. Assuming that the microbes were all destroyed, the natural pyrethrins continued to degrade despite the absence of microbes and all of the conditions relating to temperature and oxygen and moisture content. Therefore, it can be concluded that microbes are not directly involved in the degradation process.

All experiments demonstrated an initial rapid loss of pyrethrins, which was dependent on the temperature of storage. The pyrethrins content then stabilized at approximately 65% of the initial value and did not tend toward a zero concentration in the time frame of the study. Studies to elucidate this identified that the plant structure may be providing chemical or physical protection to the pyrethrins. In all experiments presented here, the majority of the degradation could be attributed to the Pyre 1 and 2 esters.

ABBREVIATIONS USED

Cin 1, cinerin I; Cin 2, cinerin II; Jas 1, jasmolin I; Jas 2, jasmolin II; Pyre 1, pyrethrin I; Pyre 2, pyrethrum II; THF, tetrahydrofuran.

LITERATURE CITED

(1) Casida, J. E.; Quistad, G. B. Pyrethrum−A Benefit to Human Welfare. In *Pyrethrum Flowers: Production, Chemistry, Toxicology and Uses*; Casida, J. E., Quistad, G. B., Eds.; Oxford University Press: New York, 1995; pp 345−350.

(2) Demoute, J. P. A Brief Review of the Environmental Fate and Metabolism of Pyrethriods. *Pestic. Sci.* **1989**, *27*, 375−385.

(3) Bayou City Mosquito Control. *Pyrethrum (A Natural Insecticide)*; http://66.51.166.210/pyrethrum.html, 2002.

(4) Head, S. W. A Study of the Insecticidal Constituents in *Chrysanthemum cinerariaefolium*. *Pyrethrum Post* **1966**, *8*, 32−37.

(5) Shene, C.; Petit-Breuihl, X.; Inostroza, S.; Bravo, S. Drying Kinetics of *Chrysanthemus cinerariaefolium* Flowers and Pyrethrin Content Decay During Drying. *Drying Technol.* **1999**, *17*, 925−935.

(6) MacIver, D. R.; Carlson, D. J. *An Overview of the Chemistry of Pyrethrins*; Pyrethrum Joint Venture/Chemical Specialties Manufacturers Association Inc.: Washington, 1996.

(7) Otieno, D. A.; Pattenden, G. Degradation of the Natural Pyrethrins. *Pyrethrum Post* **1980**, *15*, 30−37.

(8) Kawano, Y.; Yanagihara, K.; Miyamoto, T.; Yamamoto, I. Examination of the Conversion Products of Pyrethrins and Allethrin Formulations Exposed to Sunlight by Gas Chromatography and Mass Spectrometry. *J. Chromatogr.* **1980**, *198*, 317−328.

(9) Dickinson, C. M. Stability of Individual Natural Pyrethrins in Solution after Separation by Preparative High Performance Liquid Chromatography. *Pyrethrum Post* **1987**, *16*, 105−110.

(10) Brown, N. C.; Hollinshead, D. T.; Phipers, R. F.; Wood, M. C. New Isomers of the Pyrethrins Formed by the Action of Heat. *Pyrethrum Post* **1957**, *4*, 13−19.

(11) Head, S. W.; Jones, G. D. G. The Manufacture of Pyrethrum Powder from Pyrethrum Flowers Part II: Stabilisation of the Active Constituents. *Pyrethrum Post* **1965**, *8*, 14−17.

(12) Goldburg, A. A.; Head, S.; Johnson, P. Action of Heat on Pyrethrum Extract: the Isomerisation of Pyrethrins to Isopyrethrins. *J. Sci. Food Agric.* **1965**, *16*, 43−50.

(13) Gnadinger, C. B. Chapter VIII-Effect of Storage, Light, and Heat on Pyrethrum Products. *Pyrethrum Flowers*, 2nd ed.; McLaughlin Gormley King Co.: Minneapolis, Minnesota, 1936.

(14) Githinji, P. M. Thermal Degradation of Pyrethrins During Drying of Pyrethrum flowers. *pro.int.symp.dry* **1980**, *2*, 478−484.
(15) Picone, J. M. Microbial Degradation of the Natural Pyrethrins and its Implications for Pyrethrum Cropping. Honours Thesis, School of Agricultural Science, University of Tasmania, 1999.
(16) Maybe, W.; Mill, T. Critical Reviews of Hydrolysis of Organic Compounds in Water. *J. Phys. Chem. Ref. Data* **1978**, *7*, 383−415.
(17) Tattersfield, F. The Loss of Toxicity of Pyrethrum Dust on Exposure to Air and Light. *J. Agric. Sci.* **1932**, *22*, 396−417.
(18) Martin, J. T. Factors concerned in the Stability of Pyrethrum. *Pyrethrum Post* **1949**, *1*, 18−20.
(19) Chapman, R. A.; Harris, C. R.; Cole, C. Persistence of Five Pyrethroid Insecticides in Sterile and Natural, Mineral and Organic Soil. *Bull. Environm. Contam. Toxicol.* **1981**, *26*, 513−519.
(20) Glynne Jones, G. D.; Stephenson, H. Preliminary Experiments on the Storage and Subsequent Processing of Undried Pyrethrum Flowers. *Pyrethrum Post* **1959**, *5*, 13−18.
(21) Maciver, D. R. Factors Influencing the Stability of Pyrethrins in Powders. *Pyrethrum Post* **1966**, *8*, 23−26.
(22) McEldowney, A. M.; Menary, R. C. Analysis of Pyrethrins in Pyrethrum Extracts by High-Performance Liquid Chromatography. *J. Chromatogr*. **1998**, *447*, 239−243.
(23) Crombie, L. Chemistry of Pyrethrins. In *Pyrethrum Flowers: Production, Chemistry, Toxicology and Uses*; Casida, J. E., Quistad, G. B., Eds.; Oxford University Press: New York, 1995.

Received for review June 16, 2003. Revised manuscript received November 4, 2003. Accepted November 4, 2003.

JF0304425