NatSCA News

Title: A Preliminary Comparison of Trisodium Phosphate with Agepon and Decon90 as Wetting Agents to Hydrate Dried Arachnida and Myriapoda Specimens

Author(s): Janet Baccaloni

Source: Baccaloni, J. (2012). A Preliminary Comparison of Trisodium Phosphate with Agepon and Decon90 as Wetting Agents to Hydrate Dried Arachnida and Myriapoda Specimens. *NatSCA News*, *Issue 22*, 71 - 79.

URL: http://www.natsca.org/article/114

A Preliminary Comparison of Trisodium Phosphate with Agepon and Decon90 as Wetting Agents to Hydrate Dried Arachnida and Myriapoda Specimens

Janet Beccaloni

Entomology Department, the Natural History Museum, Cromwell Road, London SW7 5BD.
Email:

Abstract

An experiment was established using 98 dry Arachnida and Myriapod specimens to determine the most appropriate wetting agent solution out of Decon 90, and trisodium phosphate with Agepon. Decon 90 is an industrial cleaning agent, and Agepon is a chemical used in photographic film processing. The effects of both wetting agent solutions were varied depending on the taxa of the specimens. It was discovered that Decon 90 was most effective on the orders Scorpiones, Acari, Araneae (*Heteropoda* sp.), Amblypgygi, Solifugae and Diplopoda. Trisodium phosphate with Agepon was most effective on Opiliones and Araneae (Theraphosidae – tarantulas). Both wetting agent solutions were effective on Chilopoda.
Keywords: trisodium phosphate, Agepon, Decon 90, wetting agents, Arachnida, Myriapoda

Introduction

The Entomology Department at the Natural History Museum (NHM) has a large collection of Arachnida and Myriapoda specimens stored in 70-80% industrial methylated spirit (IMS). There is also dry, pinned material dating back to the early 19th century. Around a decade ago, there was a policy to remove type specimens from the dried collection, hydrate them using trisodium phosphate (TSP), and to store them in the main spirit collection, in order to make them more accessible and easier to study. Although this policy is no longer active, the requirement for a good hydration fluid for dried specimens still stands, as arachnologists much prefer to study hydrated dried specimens, because they are flexible, especially the genitalia.

TSP in distilled water has been used for many years for hydrating botanical, palaentological and zoological specimens (Benninghoff 1947; Van Cleave & Ross 1947). TSP and distilled water mixed with the wetting agent Agepon (used in photographic film processing) has also been used for decades, but this does not appear to have been in the public domain until recently (Jocque 2008). A wetting agent is a chemical which reduces the surface tension of a liquid, enabling the liquid to spread more easily across a solid surface, and allowing the penetration of the solid by the liquid. Indeed, this is exactly how Agepon works when mixed with TSP and distilled water – it dissolves any grease from a specimen's exoskeleton, and enables the TSP and water to be absorbed by the specimen. The TSP then swells the internal tissues and restores them to their original shape. Agepon has been renamed 'Wac wetting agent' and is manufactured by Agfa. In this digital age of photography, certain brands of wetting agent for photographic processing may become obsolete (including Wac wetting agent). But there will always be specialist film developers, albeit in much smaller numbers than before. Any photographic wetting agent, if used in the same solution as recommended by Jocque (2008) and Nellist (2009), would have the same effect on specimens (pers. comm. D. Nellist, retired chemist and ex-committee member of the British Arachnological Society).

A paper was published comparing TSP and Decon 90 as wetting agent solutions using a selection of dry, pinned arachnids and myriapods from the NHM collection (Beccaloni 2001). Decon 90 is a surface active cleaning agent, and a radioactive decontaminant used for laboratory, medical and industrial applications. It was discovered that a 2% solution of Decon 90 in distilled water was the better wetting agent solution for most orders (Beccaloni 2001). After recently receiving rave reviews for TSP with Agepon (pers. comm. R. Gabriel), it was decided to set up a similar experiment to compare TSP plus Agepon, with 2% Decon 90, to establish which wetting agent solution solution to use.

Aim

To determine the most appropriate chemical out of TSP with Agepon (A&TSP) and Decon 90, to use as a wetting agent solution on dried Arachnida and Myriapoda material.

Methods

Previously (Beccaloni 2001), only one specimen was tested per wetting agent solution and different solution strength, which was definitely not a large enough sample size. It was therefore decided to use as many specimens as possible per wetting agent solution. Ninety eight specimens without data from a selection of Arachnida and Myriapoda groups were selected - Scorpiones (scorpions), Acari (mites), Araneae (spiders), Amblypygi (whip spiders) (Fig. 1), Opiliones (harvestmen) (Fig. 2), Solifugae (camel spiders), Diplopoda (millipedes) and Chilopoda (centipedes). All of these are identified to genus or species, except the liochelid Scorpiones - see Table 1. With each group, 5 specimens from the same genus/species were used to test each wetting agent solution, except with Diplopoda, Scorpiones and Araneae. For Diplopoda, 10 specimens were used per wetting agent solution - 5 large (*Zoospherium* sp.) and 5 small (*Glomeris marginata* Villers, 1789) (Fig. 3). For Scorpiones, 7 specimens were used per wetting agent solution for Scorpiones – 2 large (*Heterometrus* sp.) and 5 medium (Liochelidae). For Araneae, 7 specimens were used per wetting agent solution - 5 medium (*Heteropoda* sp.) and 2 tarantulas (*Paraphysa scrofa* Molina) (Fig. 4).

A 2% solution of Decon 90 was used, as this was found to be the more effective strength by Beccaloni (2001). Five grams of TSP in 1L distilled water, with 5ml of Agepon was used, as this solution strength was recommended by Jocque (2008) and Nellist (2009). The specimens were fully immersed in the test chemicals in tubes or small jars (Fig. 5). Beccaloni (2001) recorded results after 17 hours immersion in the test chemicals. This approach was not ideal because several specimens did not successfully hydrate and Nellist (2009) found that successful hydration in A&TSP occurred after an immersion time of between 10 and 15 days. It was therefore decided to monitor the specimens, and remove them once they had sunk in the test chemical, which would indicate successful hydration. The deterioration of specimens is considered totally undesirable, so where specimens began to deteriorate, even when they were still floating in the wetting agent solution, they were removed. The specimens were then thoroughly washed in distilled water and transferred into 80% IMS. This resulted in various immersion times between 5.5 and 16 days.

Fig. 1. Amblypygi (whip spiders) – *Damon annulatipes* (Wood, 1869).

Fig. 2. Opiliones (harvestmen) – *Pachylus chilensis* (Guer.-Men.).

Fig. 3. Diplopoda (millipedes) – *Glomeris marginata* (Villers, 1789).

Fig. 4. Araneae (Theraphosidae) – *Paraphysa scrofa* (Molina, 1788).

Fig. 5. Centipede specimens immersed in the test wetting agent solutions.

Results

The results are presented in Tables 1 - 4, and Graphs 1 - 2. Table 1 provides details of unique specimen numbers, which were assigned for the experiment (1 to 98); specimen identification; body length; wetting agent solution used and immersion times; whether the specimens sank in IMS, and if the specimens deteriorated. Table 2 summarises the total number of specimens successfully hydrated, compared to those which partially floated (PF) or floated (F) and were still rigid, with total number of specimens which deteriorated as a result of hydration per wetting agent solution. Table 3 summarises the total number of specimens which deteriorated per order. Table 4 summarises the total number of specimens successfully hydrated, and the total number of specimens that deteriorated per order/class/subclass.

Specimen numbers	Class/ subclass/ order	Family/genus/ species ; body length	Wetting Agent	Immersion time (days)	Sunk in IMS?	Specimen deterioration
1	Diplopda	*Glomeris marginata* (Villers,1789) (10mm)	A&SP	5.5	S	Flocculation of lipids quite bad
2					F	
3					F	Flocculation of lipids quite bad
4					S	Flocculation of lipids quite bad
5					S	Flocculation of lipids quite bad
6	Diplopoda	*Glomeris marginata* (10mm)	Decon 90	5.5	S	
7					S	
8					F	
9					F	
10					F	
					S	
11	Acari	*Trombidium* sp. (10mm)	A&STP	5.5	S	
12					S	
13					S	
14					S	
15					S	Bad flocculation, white patches
16	Acari	*Trombidium* sp. (10mm)	Decon 90	5.5	S	
17					S	
18					S	
19					S	
20					S	
21	Diplopoda	*Zoospherium* sp. (40mm)	A&STP	5.5	S	Bad flocculation, started to rot and break up
22					F	Bad flocculation
23					S	Bad flocculation, covered in lipid, started to rot and break up
24					S	Started to rot and break up
25					S	Started to rot and break up
26	Diplopoda	*Zoospherium* sp. (40mm)	Decon 90	5.5	S	
27					S	Beginning to break up
28					S	Beginning to break up
29					S	Beginning to break up
30					S	
31	Scorpiones	Liochelidae (40mm)	A&STP	16	S	Starting to break up, gelatinous
32				9	S	
33				9	S	Gelatinous
34				9	S	Gelatinous
35				16	S	Starting to break up; gelatinous
36	Scorpiones	Liochelidae (40mm)	Decon 90	9	S	
37				9	S	
38				16	S	Deteriorating
39				9	S	
40				9	S	Deteriorating
41	Solifugae	*Galeodes* sp. (45mm)	A&STP	5.5	S	
42				14	S	Breaking up
43				14	S	Legs gelatinous
44				14	S	
45				5.5	PF, fl	Gelatinous
46	Solifugae	*Galeodes* sp. (45mm)	Decon 90	14	F, fl	
47				14	F, fl	
48				5.5	S	
49				5.5	S	
50				9	S	
51	Amblypygi	*Damon annulatipes* (Wood, 1869) (20mm)	A&STP	14	S	Deteriorating & breaking up
52				14	S	Breaking up
53				14	S	
54				14	S	Deteriorating
55				14	S	really beginning to deteriorate. Body contents coming out.
56	Amblypygi	*Damon annulatipes* (20mm)	Decon 90	14	PF	Body broke in half - half floated, half sank
57				14	S	
58				14	F, fl	Breaking up
59				14	PF, fl	
60				14	S	

Table 1. Results of rehydration experiment Key: F = floating; S = sunk; PF = partially floating; fl = flexible (continued overleaf)

Specimen numbers	Class/ subclass/ order	Family/genus/ species ; body length	Wetting Agent	Immersion time (days)	Sunk in IMS?	Specimen deterioration
61	Opiliones	*Gonyleptes curvipes* (Koch, 1839) (10mm)	A&STP	14	F	
62				16	F	
63				16	S	
64				16	S	
65				16	S	Breaking up
66	Opiliones	*Gonyleptes curvipes* (10mm)	Decon 90	16	F	
67				16	F	Broken up
68				16	F	Starting to deteriorate
69				16	F	
70				16	F	Breaking up
71	Araneae	*Heteropoda* sp. (Araneomorphae) (25mm)	A&STP	5.5	F, fl	
72				9	F, fl	Abdomen deteriorating
73				5.5	F, fl	Bad flocculation on specimen
74					F, fl	
75				9 9	F, fl	Abdomen very floppy
76	Araneae	*Heteropoda* sp. (Araneomorphae) (25mm)	Decon 90	14	S	Broken up
77				14	F, fl	
78				14	F, fl	Breaking up
79				5.5	S	Deteriorating
80				5.5	F, fl	Deteriorating
81	Scorpiones	*Heterometrus* sp. (110mm)	A&STP	8	PF	Deterioration; metasoma broken up; bad smell; flocculation on specimen
82				5	PF	
83	Scorpiones	*Heterometrus* sp. (110mm)	Decon 90	10	F	
84				5	PF	
85	Chilopoda	*Scolopendra subspinipes* (125mm)	A&STP	5.5	S	
86				5.5	S	
87				9	S	Flocculation on specimen
88				5.5	S	
89				5.5	S	Flocculation on specimen
90	Chilopoda	*Scolopendra subspinipes* (Leach, 1815) (125mm)	Decon 90	5.5	S	
91					S	
92					S	
93					S	
94					S	
95	Araneae	*Paraphysa scrofa* (Molina) (Theraphosidae) (55mm)	A&STP	6	PF	
96					F(fl)	floppy
97	Araneae	*Paraphysa scrofa* (Molina) (Theraphosidae) (55mm)	Decon 90	6	F	
98					F	

Table 1. (Continued) Results of rehydration experiment Key: F = floating; S = sunk; PF = partially floating; fl = flexible

wetting agent	hydrated	partially floated	floated	deteriorated
A&TSP	**42**	**1**	**6**	**27**
Decon 90	**34**	**2**	**13**	**10**

Table 2. Total number of specimens successfully hydrated, compared to those which partially floated or floated and were still rigid, with total number of specimens which deteriorated as a result of hydration.

Order/ class	Number of specimens
Scorpiones	**7 (14)**
Solifugae	**3 (10)**
Opiliones	**0 (10)**
Acari	**1 (10)**
Amblypygi	**4 (10)**
Araneae	**8 (14)**
Diplopoda	**12 (20)**
Chilopoda	**0 (10)**

Table 3. Total number of specimens showing signs of deterioration after hydration per order/class/subclass (total number of specimens tested in parenthesis).

Order/ class	A&TSP		Decon 90	
	hydrated	deteriorated	hydrated	deteriorated
Scorpiones	**5 (7)**	**5 (7)**	**5 (7)**	**2 (7)**
Solifugae	**5 (5)**	**3 (5)**	**5 (5)**	**1 (5)**
Opiliones	**3 (5)**	**0 (5)**	**0 (5)**	**0 (5)**
Acari	**5 (5)**	**1 (5)**	**5 (5)**	**0 (5)**
Amblypygi	**5 (5)**	**3 (5)**	**4 (5)**	**1 (5)**
Araneae	**7 (7)**	**5 (7)**	**5 (7)**	**3 (7)**
Diplopoda	**7 (10)**	**9(10)**	**5 (10)**	**3 (10)**
Chilopoda	**5 (5)**	**0 (5)**	**5 (5)**	**0 (5)**

Table 4. Total number of specimens successfully hydrated per order/class/subclass, with total number of specimens which deteriorated as a result of hydration (total number of specimens tested in parenthesis).

Discussion

Overall, A&TSP hydrated 42 specimens - 1 specimen partially floated after immersion in IMS, whilst 6 floated (see Table 2 and Graph 1). Decon 90 hydrated 34 specimens overall - 2 specimens partially floated after immersion in IMS, whilst 13 floated (see Table 2 and Graph 1). Initially, hydration was considered to be successful only if the specimen had sunk. However, several specimens which were partially floating or floating were actually flexible, so were considered to be successfully hydrated, given the reason for hydrating initially.

From the results, it might seem a straightforward decision as to which wetting agent solution to select, but once the deterioration of specimens is taken into account, the outcome is altered. A&TSP caused 27 specimens to deteriorate, compared to Decon 90, which affected only 10. Given that deterioration is more serious than insufficient hydration, the wetting agent solution which causes the least amount of deterioration in each case is the one recommended.

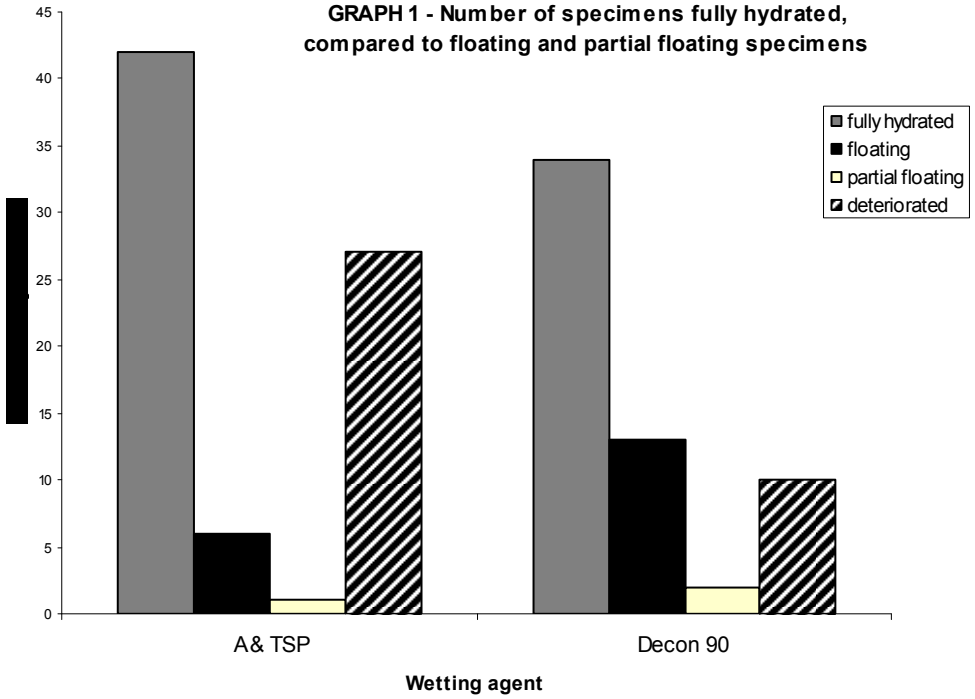
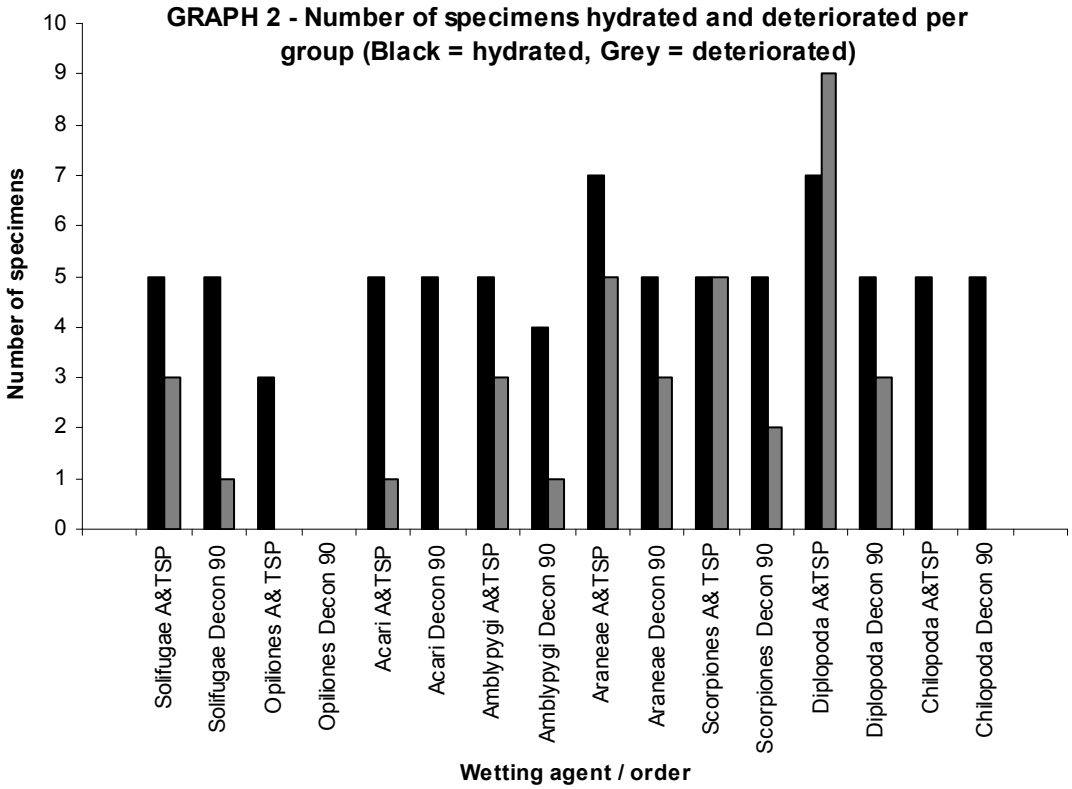


Table 3 summarises the total number of specimens showing signs of deterioration after hydration per order/ class/subclass. The three groups that showed greatest deterioration are: Scorpiones (7 out of 14 specimens), Diplopoda (12 out of 20 specimens) and Araneae (8 out of 14 specimens). It should come as no surprise that those orders with pronounced segmentation - Scorpiones and Diplopoda, should show greater deterioration. This is due to the much softer intersegmental membranes between the segments being affected more quickly than more highly sclerotized tissues. Once these tissues had split, the body contents were openly exposed, thus allowing more of the test chemical to enter the body cavity, which speeded up the hydration process and caused deterioration. Chilopods are of course, also segmented, but the specimens tested did not deteriorate. This observation has been noted in the dry collection at the NHM – no chilopod specimen has ever fallen apart, whereas dried diplopods are regularly prone to disintegration. This is because in diplopods, the soft tissue linking each sclerotised segment is thin and covers a smaller surface area compared to that in chilopods, There is no obvious explanation as to why the Araneae specimens were so affected, as they are similar in structure to solifugids, which were not so badly affected (3 out of 10 specimens).

At the group level (order/class/subclass), the performances varied greatly - see Table 4 & Graph 2. A&TSP performed fairly well with Opiliones, as it hydrated 3 out of 5 specimens, compared to no specimens hydrated by Decon 90. In addition, A&TSP caused no deterioration, so this is recommended as the wetting agent to use on Opiliones. However, it should be used with caution on other opilionid families which are much less highly sclerotized.

A&STP worked much better on tarantulas (Theraphosidae), as both specimens were flexible (even though one still floated and the other partially floated), compared to those in Decon 90, which floated and were still rigid. This concurs with information received (pers.comm. R.Gabriel). Both wetting agent solutions worked well on Chilopoda, with no deterioration, so both chemicals are recommended. This result was also noted in Beccaloni 2001.

With all the remaining orders below, Decon 90 is recommended as the preferred wetting agent, mainly due to the deterioration caused by A&TSP. With Araneae, both wetting agent solutions performed well as 12 out of 14 specimens were hydrated, although all 5 *Heteropoda* specimens floated in A&STP and 3 *Heteropoda* specimens floated in Decon 90. They were however, all flexible. It is somewhat ironic that more floated in A&STP than in Decon 90, because A&TSP is considered 'the' wetting agent solution to use (Jocque 2008; pers.comm. R.Gabriel). Although A&TSP was equally successful as Decon 90 as a wetting agent solution, it caused 5 out of 7 specimens to deteriorate, compared to 3 out of 7 with Decon 90.



With Solifugae, both wetting agent solutions hydrated all of the 5 specimens, but A&TSP caused 3 specimens to deteriorate, compared to 1 with Decon 90. With the Scorpiones, both wetting agent solutions hydrated 5 specimens each, but A&TSP caused 6 specimens to deteriorate, compared to only 2 with Decon 90. With Acari, both wetting agent solutions hydrated all of the 5 specimens, but A&TSP caused 1 specimen to deteriorate, compared to none with Decon 90. According to Beccaloni (2001), 2% Decon 90 was found to hydrate successfully 1 out of 2 acarine specimens, which is no doubt due to the shorter immersion time of 17 hours, compared to 5.5 days. With Diplopoda, A&TSP hydrated 7 out of 10 specimens, compared to 5 out of 10 with Decon 90. However, 9 specimens showed signs of bad deterioration, compared to 3 out of 10 specimens that had began to break up in Decon 90. Beccaloni (2001) found that only half of the diplopod specimens were successfully hydrated with 2% Decon 90 too, after an immersion time of 17 hours. Even by increasing the immersion time to 5.5 days, it was found to be difficult to hydrate the specimens in 2% Decon 90. For Amblypygi, A&TSP hydrated all 5 specimens, compared to 4 out of 5 with Decon 90, but 3 out of 5 specimens had begun to deteriorate, compared to 1 out of 5 specimens in Decon 90.

Due to the lack of availability of suitable material for experimentation, the sample size was too small to analyse statistically. However, it is still possible to draw useful conclusions from those data collected. It is evident that in general, *both* chemicals are a compromise, because they caused deterioration to a lesser or greater extent. This emphasises the need to only hydrate specimens where absolutely necessary.

Recommendations
Where there is an option for selectively using both wetting agents, the following is recommended:

Decon 90

- Scorpiones
- Acari
- Araneae: Araneomorphae

- Amblypygi
- Solifugae
- Diplopoda

Trisodium phosphate with Apegon

- Opiliones
- Araneae: Theraphosidae

Either

- Chilopoda

Further Work

The above data highlight the need for further, extensive tests on a much greater number of specimens, so that the data can be statistically analysed. However, in practise, it is very difficult in to obtain so many specimens for experimental purposes.

Since the work in this paper was undertaken, it has come to light that the problem of specimen deterioration can be prevented by warming the wetting agent solutions to around 50ºC. This catalyses the reaction and reduces the need to leave the specimens in the solutions for such long periods. The use of an air vaccum pump, which removes trapped air from the specimens and draws fluid in, can be used alongside the heating methodology (pers. comm. Simon Moore). It is therefore proposed that another experiment is run, with warmed wetting agent solutions and the use of a vacuum air pump, in order to perfect the wetting agent technique. In addition, a study would also be undertaken to establish whether the DNA of the specimens is adversely affected by such hydration of the tissues.

Acknowledgements:
Thank you to the following for their help and advice; David Nellist, Simon Moore, Ray Gabriel, Amoret Spooner, Darren Mann.

Bibliography

Beccaloni, J. (2001) A comparison of trisodium phosphate and Decon 90 as hydrating agents for Arachnida and Myriapoda dry specimens. *The Biology Curator*, 22:15-23.

Benninghoff, W.S. (1947) Use of trisodium phosphate with herbarium material and microfossils in peat. *Science*, 106:325-326.

Jocque, R. (2008) How to hydrate dried spiders. *Newsletter of the British Arachnological Society*, 112:5.

Nellist, D. (2009) The identification of desiccated adult spiders in a collection supposedly made by A.R.Jackson in the late 1930s at Wheatfen Broad, Norfolk. *Newsletter of the British Arachnological Society*, 115:12-13.

Van Cleave, H.J., Ross, J.A. (1947) A method for reclaiming dried zoological specimens. *Science*, 105:318.