THE TOLERANCE OF THREE STEINERNEMATID NEMATODES TO DESICCATION AND THE EFFECTS OF SEVERAL TYPES OF ADDITIVES ON THEIR MOBILITY

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BY

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ABSTRACT

This was a two-part study. In the first part, differences in the ability of three Steinernematid nematodes to remain mobile at various saturation deficit levels were investigated. A TI_{50} value (time required for 50% of the nematodes within a sample to reach immobility) was obtained for each saturation level and each species. In the second part, the potential of 11 types of additives to enhance nematode mobility (increase the TI_{50} value) at various saturation deficit levels was evaluated.

Results indicated that each species had significantly different TI_{50} values for each saturation level. *Steinernema feltiae* (All strain), *Steinernema* sp. Nfld. strain No.1(L1C strain), and *Steinernema bibionis* had the highest, medium and lowest values, respectively. The additives tested separated into groups based on their TI_{50} values. The best group with the highest TI_{50} values consisted of base, heavy and 2251 additives. The second group, klearol, pyronyl and Exxon, and the third group, soybean, savol, cottonseed, sunspray and citrus, had the median and lowest TI_{50} values, respectively. The additives of the first group possessed the lowest relative viscosity values, while those of the second and third groups possessed the median and highest viscosity values, respectively.

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INTRODUCTION

The widespread use of chemical insecticides over the past several decades has come under close scrutiny by the scientific community, as well as the general public. Government agencies are, consequently, seeking safe alternatives to chemical sprays. One approach is the concept of Integrated Pest Management (IPM), whereby major emphasis has been placed on the reduction of chemical sprays by the introduction of biological control agents. Entomophilic nematodes are one group of biocontrol agents which may have potential in controlling major agricultural, orchard and forest insect pests (Poinar, 1983; Nickle, 1984). Several recent reviews have evaluated these nematodes as biological control agents for many insect pests (Webster, 1980; Gaugler, 1981; Nickle, 1984 and Kaya, 1985). Nematodes of the genus *Steinernema* possess unique characteristics favoring their use as biological control agents. These nematodes are able to actively seek, penetrate and kill their hosts. Host mortality is due to the associated symbiotic bacterium, *Xenorhabdus nematophilus* (=*Achromobacter nematophilus*), located within the esophageal region of the third stage juvenile or infective stage. This bacterium is released into the hemolymph of the host insect after penetration by the nematode. The host usually dies within 24 to 48 hours by septicemia and the nematodes are nourished by the products of bacterial breakdown of the host cadaver (Wouts, 1984).

Although these nematodes can effectively kill their hosts in the laboratory, field attempts to control various agricultural, orchard and forest insect pests by foliar spraying have yielded inconsistent results. In many instances, the most probable cause of their low field efficacy has been the rapid desiccation of the infective stages due to low moisture levels (Moore,1973; Simons,1973; Simons and Poinar,1973; Wouts,1984). Several researchers have stressed the importance of maintaining high humidity levels, in excess of 90%, to ensure significant infection by nematodes and insect control (Welch and Briand,1961a,b; Moore,1965; Kamionek *et al.*1974). Attempts have been made to

reduce desiccation and thus enhance nematode efficacy by the addition of various gels (Webster and Bronskill,1968; Nash and Fox,1969) and oils (Bedding,1976). These attempts, however, were unsuccessful at increasing the efficacy of the nematode and thus the control of insect pests (Gaugler,1981). Recently, Shapiro *et al.* (1985), evaluated a wide array of potential antidessicants such as oils, spreaders, and wetting agents, in the laboratory, but their effectiveness in the field has not yet been evaluated.

The present study examined the differences between one species and two strains of nematodes in relation to duration of activity under various saturation deficits and the effect of various additives on enhancing the duration of activity under several saturation deficit levels. The nematodes investigated were *Steinernema bibionis* (Bovien, 1937), *S. feltiae* (All strain), (Filipjev, 1934) and *Steinernema* sp. Nfld. strain No. 1 (L1C strain). It was hypothesized that certain additives could coat the infective stages, thereby, reduce the rate of desiccation in unsaturated air and enhance the period of mobility of the infective stage. The aim was to determine which nematode showed the most promise for application under a specific saturation deficit and whether certain additives could enhance nematode mobility.

HISTORICAL SURVEY

Travassos (1927) redescribed *Aplectana kraussii* Steiner 1923 and placed it in the new genus *Steinernema*. Later, Filipjev (1934) placed it at the subfamily level and then Chitwood and Chitwood (1937) placed it at the family level, Steinernematidae. When Steiner (1929) described the genus *Neoaplectana*, he separated it from the genus *Steinernema* by the number of cephalic and genital papillae. However, Mracek (1977) in a redescription of *S. kraussii*, found a similarity in the distribution of cephalic papillae with that of *Neoaplectana*. Recently, Wouts *et al.* (1982) proved that the number of cephalic papillae were the same, hence the two genera are synonymous. Currently, the genus name of *Steinernema* is accepted as the proper nomenclature.

The significance of a symbiotic bacterium in the life cycle of the nematode was first suggested by Bovien (1937) and later confirmed by Dutky and Hough (1955). The bacterium was first described by Poinar and Thomas (1965) as *Achromobacter nematophilus* and later redescribed by Thomas and Poinar (1979) as *Xenorhabdus nematophilus*.

The first attempt to control insect pests by use of the entomophilic nematode, *S. glaseri*, was against the soil-inhabiting larvae of the Japanese beetle, *Popilla japonica* Newm. (Glaser, 1932). Preliminary tests indicated that this nematode killed insects quickly in the laboratory; hence, subsequent experiments were carried out to test its potential in the field (Glaser, 1931, 1940). Initial results were encouraging, but subsequent applications failed to achieve adequate control (Glaser, 1932; Glaser and Farrell, 1935).

The discovery of a *S. feltiae* population in the codling moth, *Cydia pomonella* (L.), in the United States (Dutky and Hough, 1955; Dutky *et al.* 1962) restimulated interest in the possibility of using nematodes as biocontrol agents and subsequent work

by Dutky (1968) led to the development of a spray application that gave 60-70% control of the codling moth in the field. In many cases, however, nematodes were applied in habitats in which the infectives could not survive (Gaugler,1981).

The use of nematodes as biocontrol agents against insects in protected habitats such as soil (Kaya,1985) and within insect galleries, seeds and tree fruits (Gaugler,1981) has shown higher potential for control. Soil application of *S. feltiae* showed considerable promise in controlling rootworm larvae, *Diabrotica* sp.in corn (Poinar *et al.*1983). *Steinernema feltiae* and *S. glaseri* provided 65% mortality in the field of the strawberry root weevil, *Nemocestes incomptus*, infecting raspberry plantings (Georgis and Poinar, 1984). Against the pecan weevil, *Curculio caryae* (Horn), *S. feltiae* yielded a larval mortality of 67%, which was higher than that achieved with three different fungal pathogens (Tedders *et al.*1973). In Australia, a large scale application of *S. bibionis* killed over 90% of blackcurrant borers, *Synanthedon tipuliformis* (Clerck), within their galleries (Bedding and Miller,1981; Miller and Bedding,1982). In commercial fig orchards, the carpenterworm, *Prionoxystus robinae*, was totally eradicated by applications of *S. feltiae* (Lindegren and Barnett,1982; Lindegren *et al.*1981). Applications of *S. feltiae* to control the navel orangeworm, *Ameyelois transitella*, a serious pest of almonds, resulted in 100% mortality in one test and a 55% reduction in another, which provided a 34% overall reduction in almond damage (Lindegren *et al.*1978).

The application of nematodes against foliar feeding insect pests has met with the least success due to poor nematode survival. Zelazny (1985) reported that *S. feltiae* failed to reduce population levels of two coconut pests, *Oryctes rhinocerus* L. and *Tirathaba rufivena*. Field trials also showed that a strain of *S. feltiae*, known as DD-136, did not control larval populations of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Welch and Briand,1961a). Chamberlain and Dutky (1958) showed that significant larval reduction of the tobacco budworm, *Heliothis virescens* (Fab.), only occurred after rains or high humidity when *S. feltiae* was applied to the leaves. Similarly, *S. feltiae* was not

able to control apple defoliators because of the rapid desiccation of the infective stages on the leaves (Jaques,1967). These studies have indicated the importance of adequate moisture levels to maximize nematode survival when applied against foliar feeding insect pests.

During the past decade, research has been directed on the use of evaporetardants in extending nematode persistence in the field during the post-treatment period. In a study by Kaya *et al.* (1981), infective stages of *S. feltiae*, immersed in 2% Volck^{R} oil, did not significantly reduce populations of Western spruce budworm, *Choristoneura occidentalis* Freeman, or elm leaf beetle, *Pyrrhalta luteola* (Muller), when applied in the field. However, field tests of the antidesiccants Methocel^{R}, a methycellulose polymer; Folicote^{R}, an evaporetardant; Norbak^{R}, a transpiration reducer on seedlings; and Nalcotrol^{R}, an antidrift agent, increased the percent infection of *S. feltiae* in the Colorado potato beetle, *Leptinotarsa decemlineata* Say, from 10% in water alone to 30 to 60% (MacVean *et al.*1982). Similarly, the addition of Folicote^{R}, Gelgard M^{R}, a water thickener, and Arlatone^{R}, a surfactant, to an aqueous solution containing *S. feltiae* increased the average mortality of larch sawfly larvae, *Pristiphora erichsonii* (Htg.), from 24% to 90% (Webster and Bronskill,1968). The use of glycerin, Emgard 2050^{R}, Sole-onic CDS^{R} and IGEPON AP-78^{R} as wetting agents did not provide sufficient control of the Nantucket pine tip moth, *Rhyacionia frustrana* (Comstock), to recommend its use (Nash and Fox,1969). No significant differences were found in infection levels of European elm bark beetle, *Scolytus scolytus* (Fab.), when sprayed with the DD-136 strain of *Steinernema* sp. immersed in 10% glycerin and in distilled water alone (Finney and Walker, 1977; Finney, 1984).

The past research indicates the complexities involved in using entomophilic nematodes as biocontrol agents of various agricultural, orchard and forest insect pests. Among these complexities, the reduction of water loss during the post-spray period is probably the limiting factor in using entomophilic nematodes as biocontrol agents for insect pests.

MATERIALS AND METHODS

The one species and two strains of nematodes investigated throughout this study were *Steinernema bibionis*, *Steinernema* sp. Newfoundland strain No. 1 (L1C strain) and *Steinernema feltiae*, (All strain). Throughout the remaining portion of this thesis, these nematodes will be referred to as *S. bibionis*, L1C strain and All strain. The infective larval stage of these nematodes were propagated by passage through larvae of the greater wax moth, *Galleria mellonella* (L.), using the technique described by Dutky *et al.* (1964). Infective larvae were stored in distilled water at 5°C until required. Infective larvae were not stored for greater than two months and were checked for mobility by observing the nematodes at 20°C before use.

PART I. Desiccation Exposures

A. The Desiccation Chamber

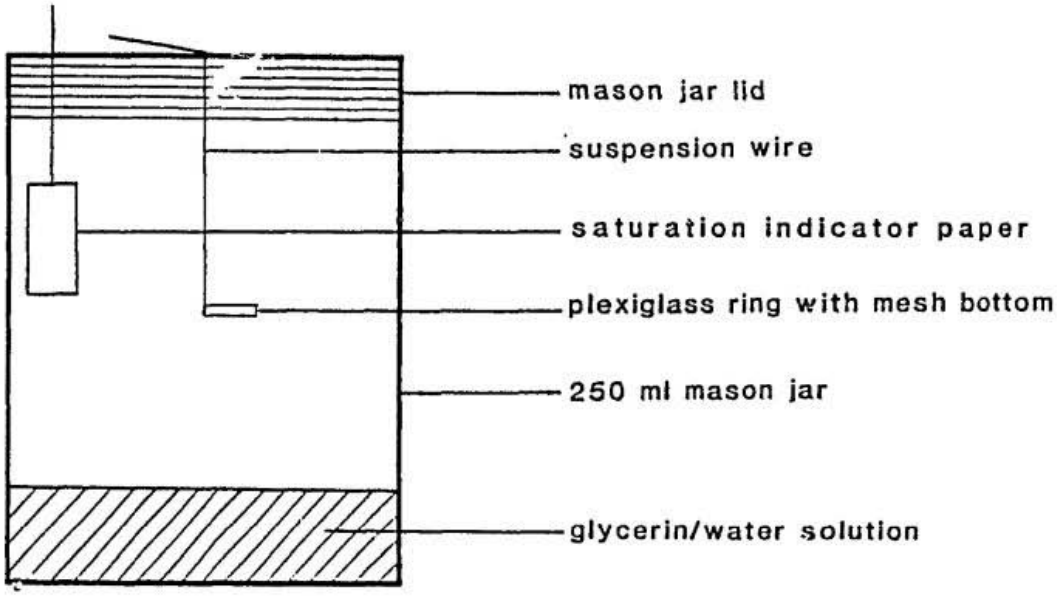
A 250ml mason jar was modified and used as a humidity chamber; several of these chambers could be made with ease in a short time for simultaneous use (Figure 1). The lid of the jar was modified by removing its center and replacing it with two layers of 100% latex dental dam material (Dental Supplies, St.John's, NF). A 3 cm slit in each layer in a cross pattern allowed penetration into the chamber with minimal interruption of the internal saturation level. The dental dam material was glued to the rim of the lid to make an airtight seal and also to make the lids reuseable.

B. Maintenance of Saturation Levels

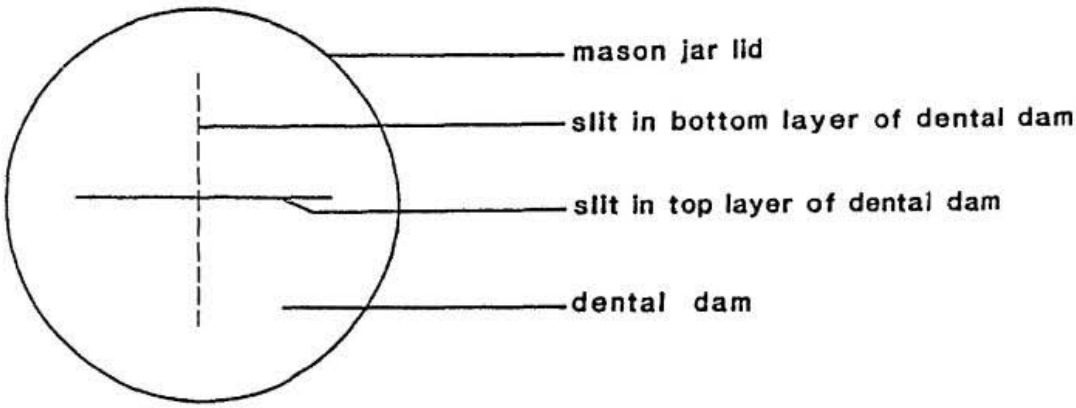
Saturation levels were maintained using mixtures of glycerin and water to yield a specific saturation level (Johnson,1940). Appendix A outlines the various proportions

Figure 1. Side view and top view of a humidity chamber made from a 250ml mason jar.

SIDE VIEW



TOP VIEW



of glycerin and water to yield specific saturation levels. After thoroughly mixing the glycerin and water required, the lid was placed on the jar and sealed with Parafilm^R to prevent air leakage. Saturation levels were allowed to stabilize for 24 hours before use. Indicator papers hung on the inside of each chamber and compared to a color code, indicated the presence of a desired saturation level. These papers were impregnated with equal volumes of 50% w/v cobalt chloride ($CoCl.6H_2O$) and 25% w/v potassium thiocyanate (KCNS) (Solomon, 1944). The color standards for matching test papers were prepared by exposing impregnated papers in constant saturation levels and then sealing them in vials containing liquid paraffin. A total of seven saturation deficits, 23.4, 19.4, 15.5, 13.1, 8.8, 6.2 and 3.5 millibars, were tested in this part of the study.

C. Exposure Method

The apparatus designed to suspend the nematodes within the humidity chamber consisted of a piece of Nitex^R screening (100% nylon; 10um pore size; (B.S.H. Thompson & Co. Ltd., Quebec) attached to a glass plexiglass ring (diameter 1.5cm; height 3mm) using chloroform. Using an insect pin bent at the tip, ten infective stage nematodes were individually removed from a stock supply and placed within the ring which was partially immersed in distilled water. Excess water within the ring was removed by blotting dry on a Whatman No. 3 filter paper. Using a piece of innoculating wire attached to the ring, the apparatus was carefully manipulated into the chamber through the pair of slits in the dental dam material. After a predetermined exposure time interval of 10 minutes, the ring was carefully removed from the chamber and immediately flushed with distilled water into a gridded petri dish to check for nematode mobility. Mobility was detected by movement of the nematode after probing with an insect pin. Exposures were carried out in total darkness at 20°C.

A mobility curve was plotted for each species exposed at each saturation level. Each exposure was repeated 5 times with 10 nematodes per replicate. The number of predetermined time intervals used depended on the time required to reach 100%

immobility. From the resulting mobility curve, a TI_{50} (time at which 50% of the nematodes became immobile) value was determined. A TI_{50} curve was then plotted for each species over the seven saturation levels.

PART II. Antidesiccant Exposures

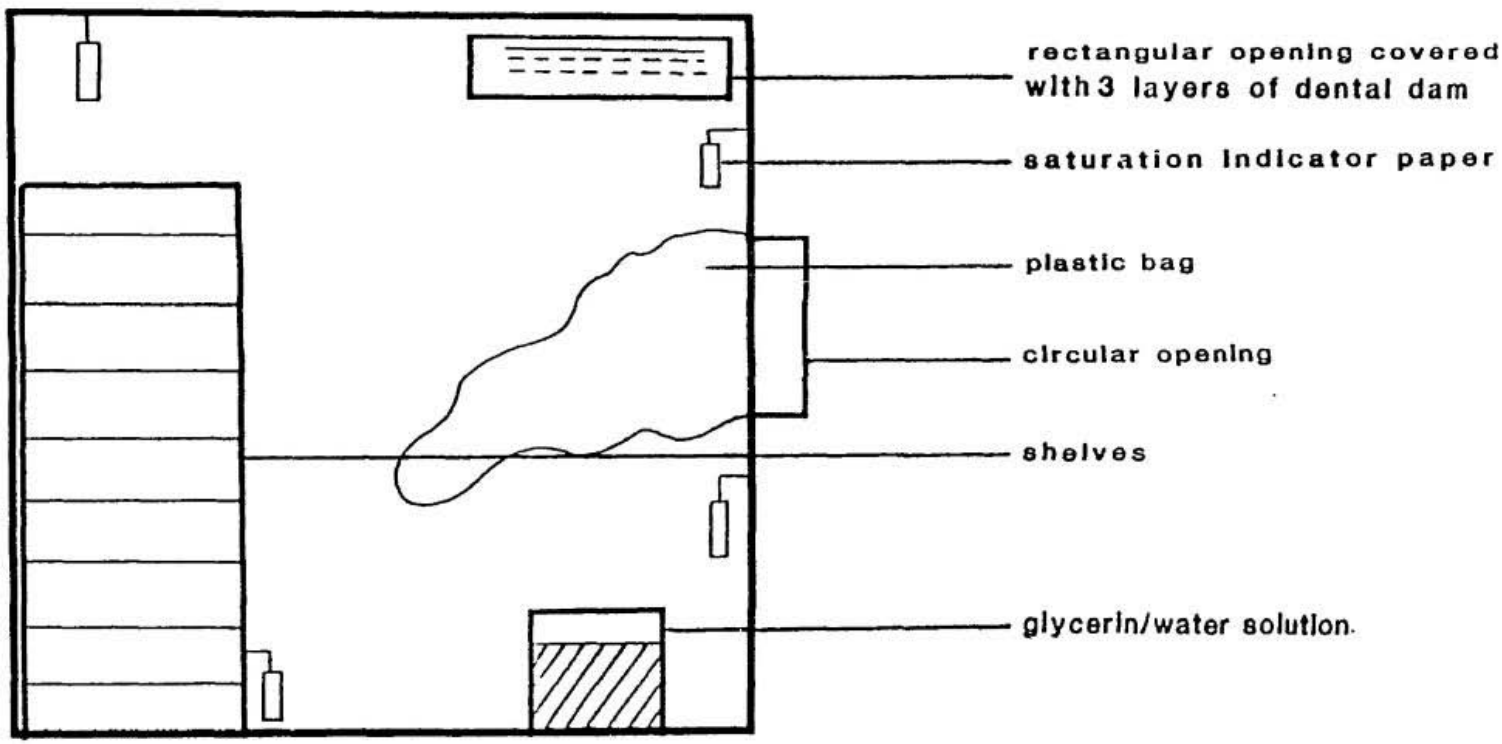
A. The Humidity Chamber

A larger chamber was designed for this part of the study to allow several samples to be exposed simultaneously with minimal interruption of the internal saturation level. The chamber was constructed of plexiglass and had a volume of approximately $0.027m^3$ (Figure 2). On one side of the chamber, a circular portion (dia. 16cm) was removed and replaced with a plastic bag sealed around the edge to make an airtight fit. This bag allowed manipulation of objects within the chamber without any interruption of the internal saturation level. A rectangular opening (2.5cm x 15cm) was made in the upper left side of the chamber and was covered with 3 layers of dental dam material. In each layer a longitudinal slit (length 10cm) was cut with each slit being off center to the one underneath. This opening permitted access to the inside of the chamber with minimal interruption . Test tube racks were placed inside the chamber to serve as shelves for petri dishes containing samples.

B. Maintenance of Saturation Levels

Saturation levels were maintained in the chamber by two 400ml solutions of glycerin and water, as previously described, contained in two deep-well dishes. A large volume of solution was used due to the large volume of the chamber and also to maintain a specific saturation level for a longer period of time. Several humidity indicator papers were hung in the chamber to indicate a uniform saturation level throughout the chamber.

Figure 2. Side view of plexiglass humidity chamber.


rectangular opening covered
with 3 layers of dental dam
saturation indicator paper
plastic bag
circular opening
shelves
glycerin/water solution

C. Method for Testing Additives

Using a calibrated micropipette, five 2ul droplets containing water, additive and infective stage nematodes, were placed in a small petri dish (dia. 3cm). Each droplet had concentration of 10% additive and 90% distilled water. Each petri dish containing 5 droplets was placed within the chamber at a particular saturation level and its starting time recorded. After a 30 minute interval, the dish was removed and each droplet was examined under a dissecting microscope to determine nematode immobility. If 100% immobility was not reached within a particular time interval then another dish containing 5 droplets was exposed for 60 minutes and then removed from the chamber for examination. A repetition of this step would continue, increasing the time interval for the exposure by 30 minutes until 100% immobility was reached in all five droplets. Each time interval was repeated twice for a total of ten droplets per exposure time. All exposures were tested at saturation deficits of 19.4, 8.8 and 3.5 millibars and at a temperature of 20°C. The additives tested were oils of Soybean, Cottonseed, Savol, Citrus, Sunspray, Pyronyl, Orchex Exxon, Klearol, Base, Heavy and 2251. These were obtained from Dr. Martin Shapiro of the Otis Methods Development Center, U.S.D.A, Otis ANGB, Massachusetts 02542. Appendix N gives, as far as is available, a description of the specifications of each of these additives. It should be noted that the information on some of the additives is either brief or is missing due to the reluctance of various companies to give information on their products.

PART III. Measurement of Additive Viscosities

The relative viscosity of each additive was measured by recording the time required, in seconds, for 9mls of the additive to dispense from a 10ml glass pipette. This procedure was replicated three times for each additive in order to calculate a mean dispensing time; the temperature was held constant at 23°C during the procedure. In order

to eliminate variation which may occur by using different pipettes for each replicate, the same pipette was used. After each replicate, the pipette was thoroughly cleaned and aerated to completely dry it.

PART IV. Statistical Analysis

Probit analysis (SAS Institute, 1985) was used to transform the percentage data in Parts I and II as indicated in Tables 1 and 2, respectively. Scheffe's method (Zar, 1974) (alpha = 0.01) was used to determine whether any significant differences existed within species and saturation deficits (Part I) and between the relative viscosities of the additives (Part III). The nonparametric method of Kruskal-Wallis (Zar, 1974,) (alpha = 0.05) was employed in Part II to determine whether any significant differences existed within species, saturation deficits and additives. Tables 3, 4 and 5 outline the test results using the Kruskal-Wallis procedure.

RESULTS

PART I. Desiccation Exposures

The mean (X) percent (%) immobility of *S. bibionis*, L1C strain and All strain, exposed for predetermined time intervals over the seven saturation deficit levels is shown in Figures 3,4 and 5 respectively. Each observed point on the curves, as depicted in Figures 3,4 and 5, is based on 5 replicate exposures with 10 nematodes per exposure. Appendices B,C and D numerically summarize these points giving their means (X) and standard errors (SE) for each saturation deficit level and species. As the saturation deficit decreased, the time required to reach 100% nematode immobility increased as indicated by a shift in the curves to the right. The response to desiccation at high saturation deficits is rapid, as indicated by the near vertical slopes for curves A,B,C,D,E,F and G for each species. The horizontal distance between the curves increases abruptly when the 6.2 millibar saturation deficit is reached as depicted after curve F for each species.

A mean TI_{50} value was derived from the curves in Figures 3,4 and 5. Table 1 outlines each of these mean TI_{50} values for each saturation deficit and species. Figure 6 shows the response for each species by the mean TI_{50} value for each saturation deficit level. *S. bibionis*, L1C and All strain had the lowest, medium and highest TI_{50} values, respectively, under each saturation deficit tested (Scheffe's test, alpha = 0.01). Scheffe's test (alpha = 0.01) indicated that the TI_{50} values for each species are significantly different under each saturation deficit level. As indicated previously, the abrupt change in response when the 6.2 millibar saturation deficit level is reached is also apparent in Figure 6 whereby the curves have a tendency to "flatten out" when the highest saturation level is reached.

Figure 3. The mean percent immobility of *S. bibionis* exposed at various saturation deficits (millibars) for predetermined time intervals. (Note: X-axis intercepts represent observed points. Curves are fit by eye for illustrative purposes. A - 23.4 millibars, B - millibars, C - 15.5 millibars, D - 13.1 millibars, E - 8.8 millibars, F - 6.2 millibars, G - 3.5 millibars.)

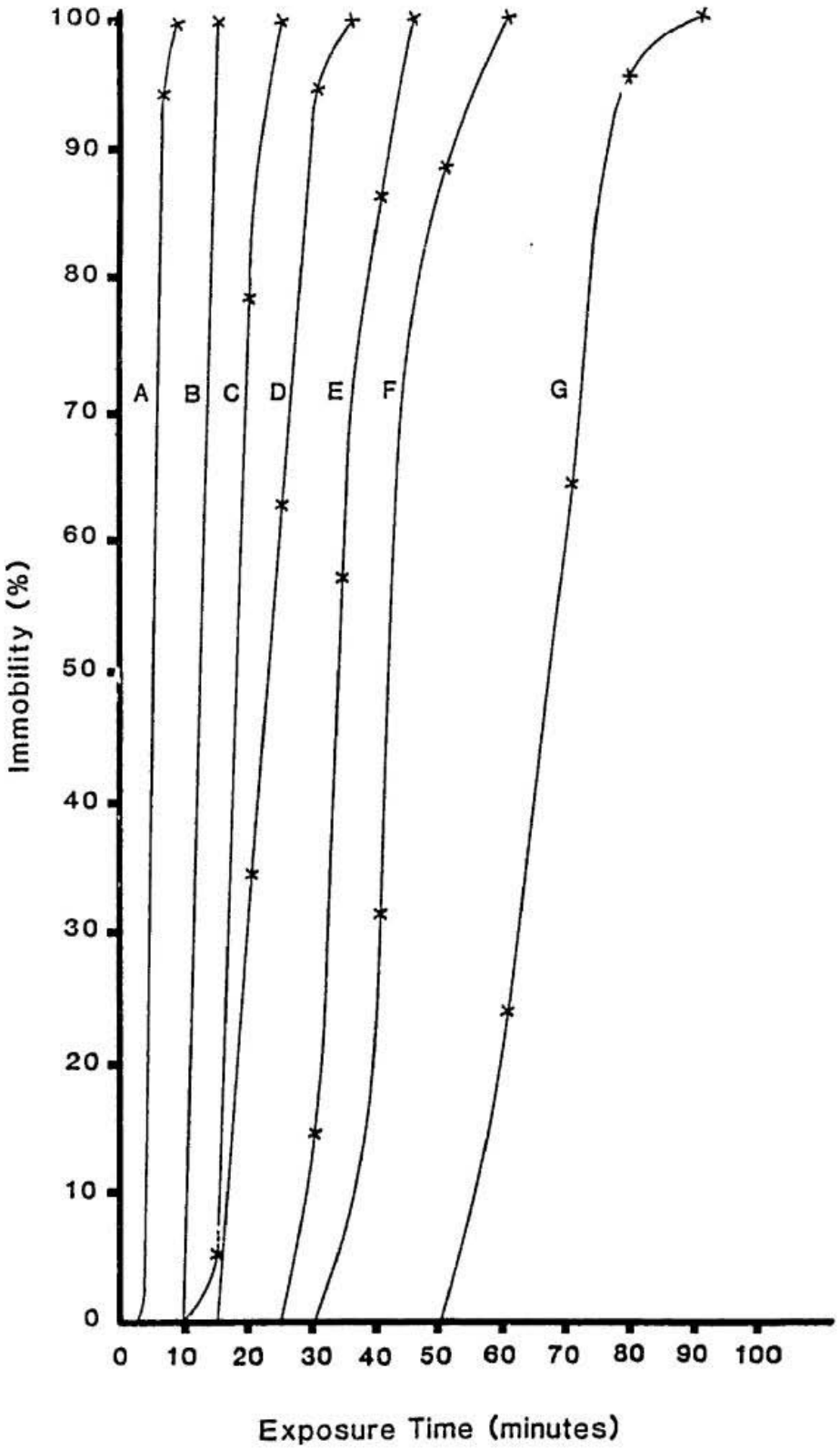

100
90
80
70
60
50
40
30
20
10
0
Immobility (%)
A
B
C
D
E
F
G
0
10
20
30
40
50
60
70
80
90
100
Exposure Time (minutes)

Figure 4. The mean percent immobility of L1C exposed at various saturation deficits (millibars) for predetermined time intervals. (Note: X-axis intercepts represent observed points. Curves are fit by eye for illustrative purposes. A - 23.4 millibars, B - millibars, C - 15.5 millibars, D - 13.1 millibars, E - 8.8 millibars, F - 6.2 millibars, G - 3.5 millibars.)

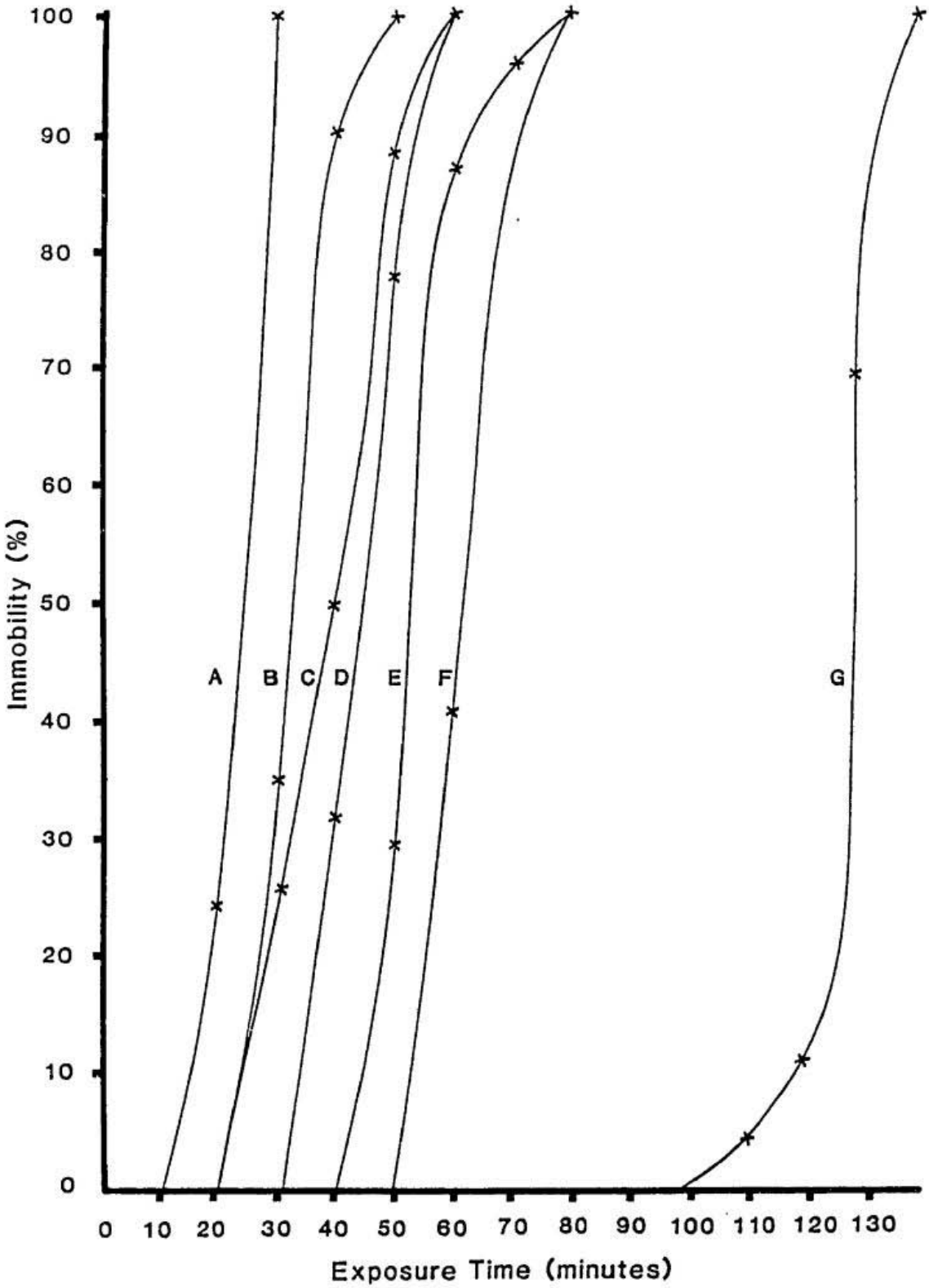

Immobility (%)
100
90
80
70
60
50
40
30
20
10
0
A
B
C
D
E
F
G
0 10 20 30 40 50 60 70 80 90 100 110 120 130
Exposure Time (minutes)

Figure 5. The mean percent immobility of All strain exposed at various saturation deficits (millibars) for predetermined time intervals. (Note: X-axis intercepts represent observed points. Curves are fit by eye for illustrative purposes. A - 23.4 millibars, B - millibars, C - 15.5 millibars, D - 13.1 millibars, E - 8.8 millibars, F - 6.2 millibars, G - 3.5 millibars.)

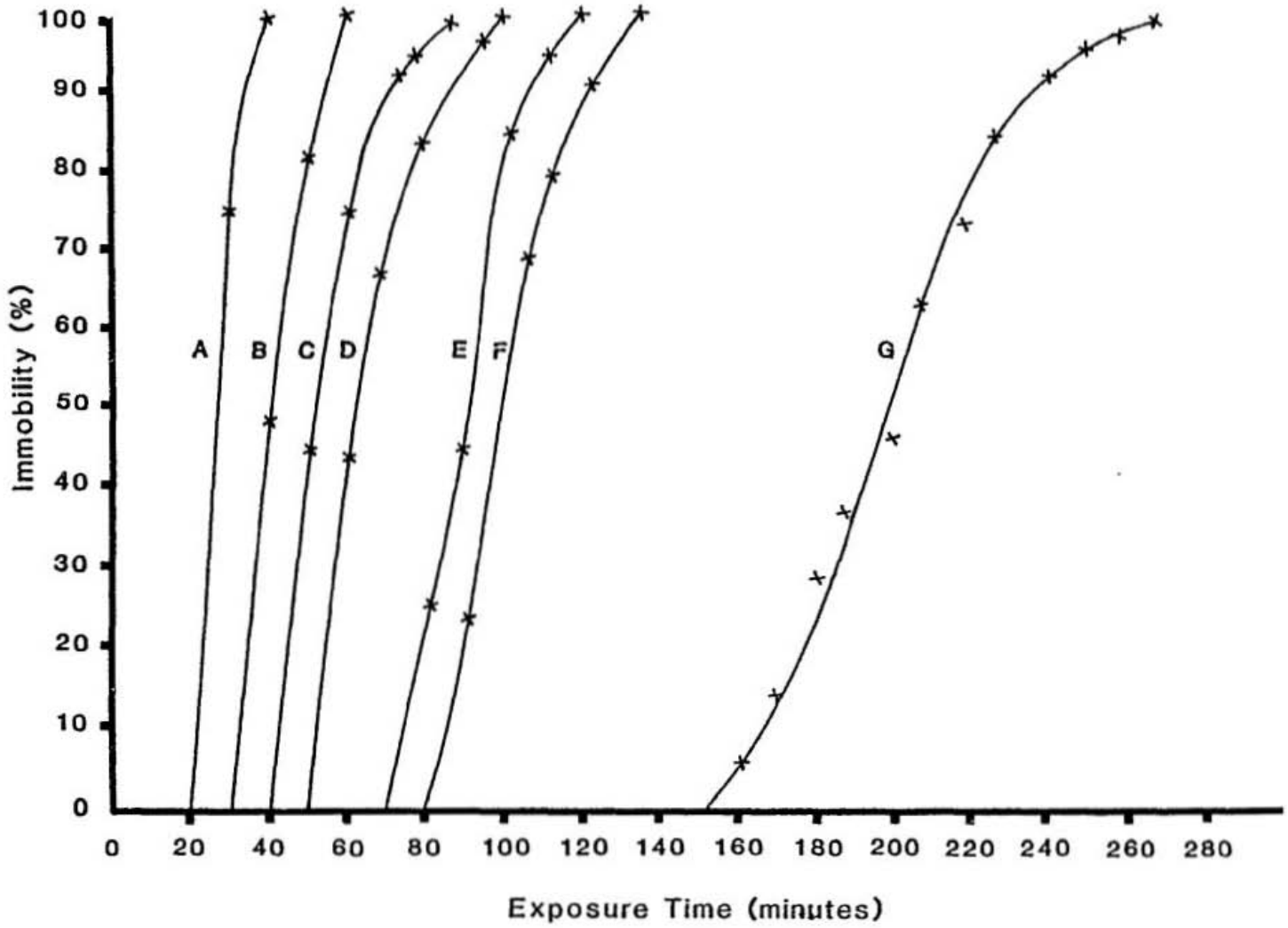
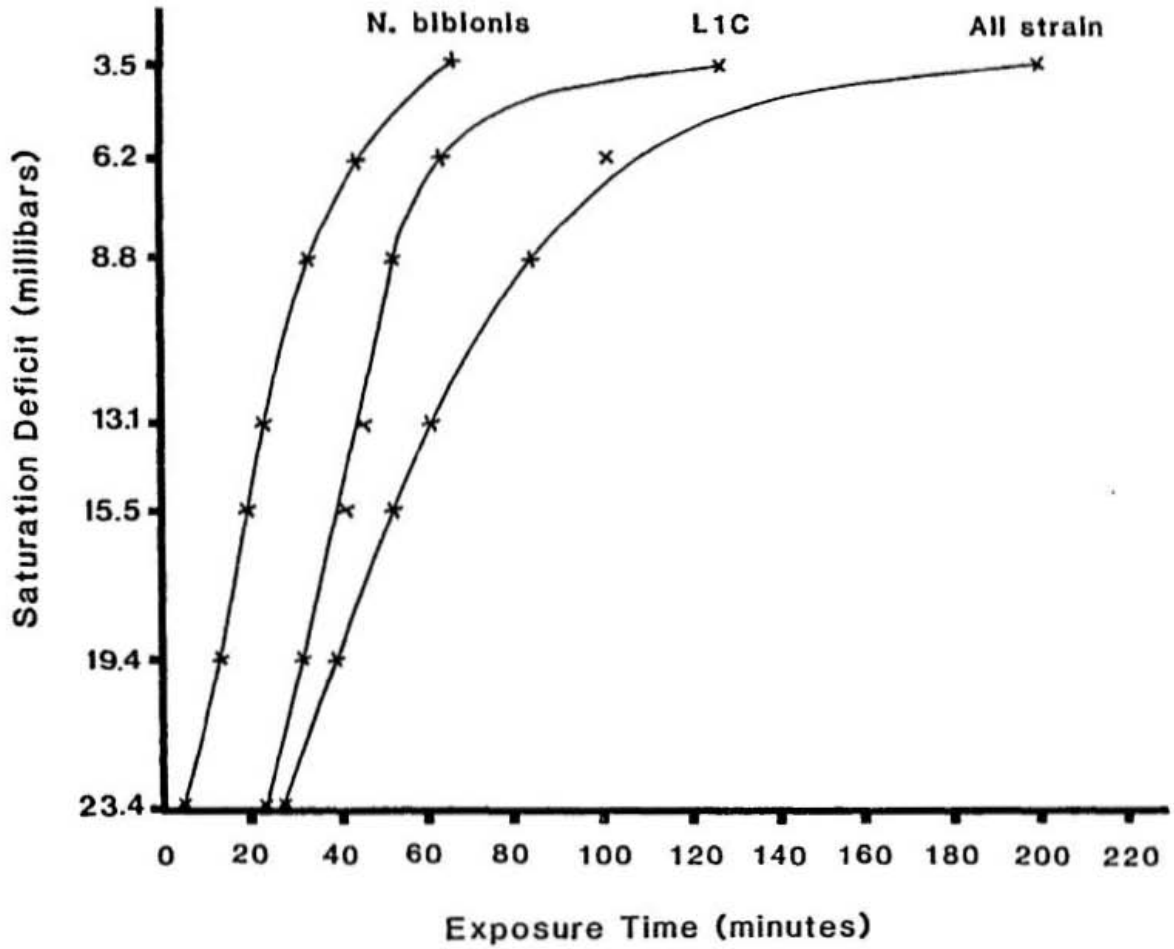

Immobility (%)
100
90
80
70
60
50
40
30
20
10
0
A
B
C
D
E
F
G
0
20
40
60
80
100
120
140
160
180
200
220
240
260
280
Exposure Time (minutes)

Table 1. The mean TI_{50} values (in minutes), determined by probit analysis, of *S. bibionis*, L1C and All strain exposed at various saturation deficits (millibars) for predetermined time intervals.*

Saturation Deficit (millibars)	Species		
	S. bibionis	L1C	ALL Strain
23.4	**6.5** (3.8–10.3)	**21.4** (20.8–22.1)	**28.1** (26.7–28.5)
19.4	**14.1** (12.5–15.4)	**31.5** (30.3–32.8)	**41.6** (40.2–43.1)
15.5	**17.0** (15.9–18.2)	**37.6** (36.0–39.2)	**53.2** (51.2–55.1)
13.1	**21.8** (21.0–22.6)	**43.5** (42.3–44.7)	**65.0** (63.6–66.5)
8.8	**34.6** (33.7–35.4)	**53.7** (52.6–54.7)	**89.3** (87.9–90.6)
6.2	**42.7** (41.8–43.6)	**62.6** (61.1–64.2)	**98.9** (97.5–100.3)
3.5	**65.7** (64.2–67.2)	**127.1** (124.7–129.5)	**198.6** (195.9–201.2)

* Upper and lower confidence limits are shown in parentheses under each mean.

Figure 6. The mean TI_{50} values of *S. bibionis*, L1C and All strain exposed at various saturation deficits (millibars) for predetermined time intervals. (Note: Curves are fit by eye for illustrative purposes. X-axis intercepts represent observed points.)

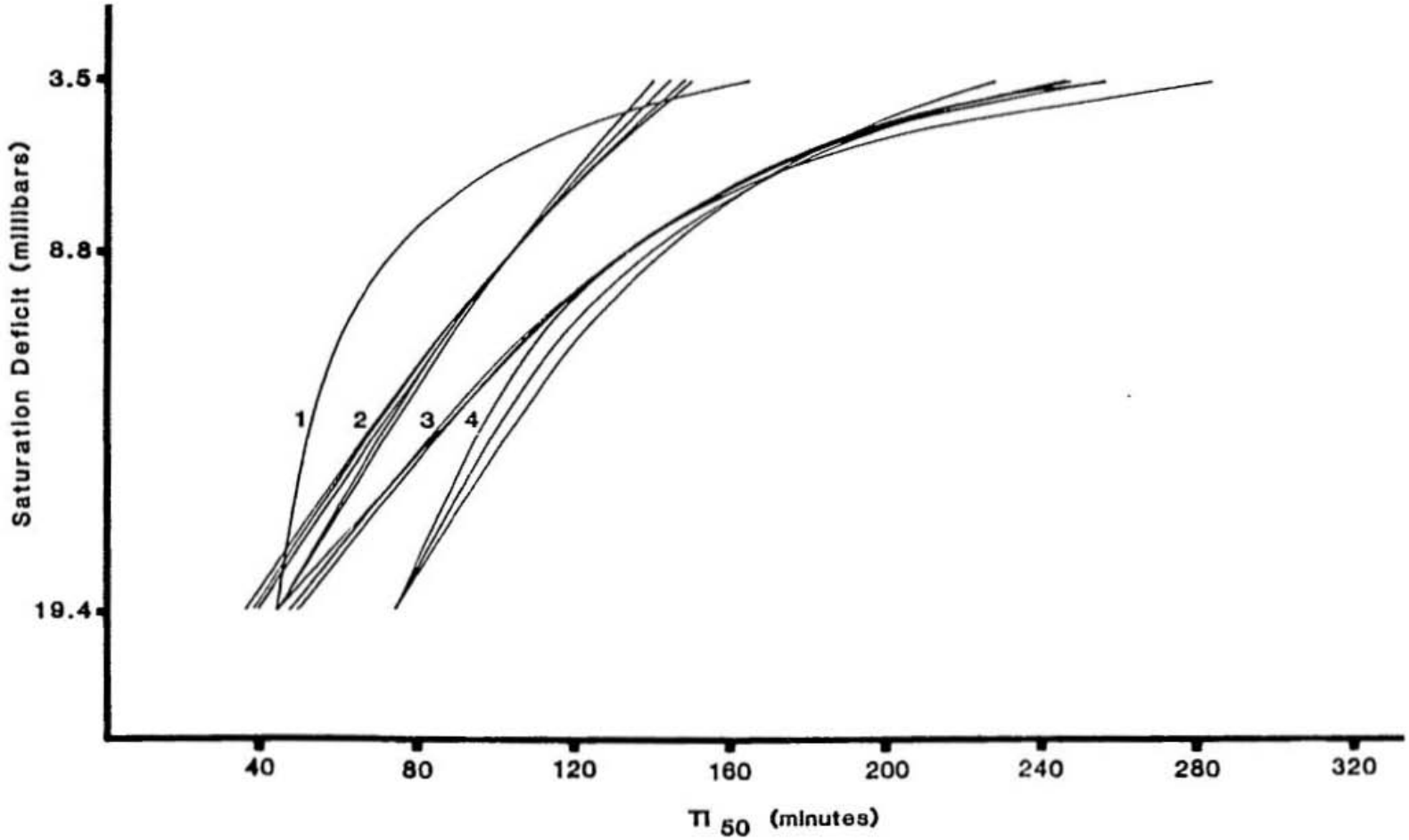

N. bibionis
L1C
All strain
3.5
6.2
8.8
13.1
15.5
19.4
23.4
Saturation Deficit (millibars)
0
20
40
60
80
100
120
140
160
180
200
220
Exposure Time (minutes)

PART II. Exposures Using Additives

Figures 7 to 9 depict the mean TI_{50} values in response to each additive tested at each of three saturation deficit levels for each species. Although, these curves may lack clarity in showing the response for each individual additive, they serve to indicate the grouping effect of all the additives based on their similar TI_{50} values. Hence, due to the crowded nature of these curves, the observed points are not indicated on the curves but instead are summarized in Table 2 for each additive, saturation deficit level and species. The TI_{50} values outlined in Table 2 are the result of the data transformation procedure known as Probit Analysis (SAS Institute, 1985). The corresponding confidence limits for each TI_{50} value are outlined in Appendices Y_1, Y_2 and Y_3. The mobility response curves used to derive Figures 7, 8 and 9 are given in Appendices E to M. The observed points along the curves in these appendices are summarized in Appendices O to W.

The Kruskal-Wallis test (alpha = 0.01) was used to detect whether any significant differences occurred between saturation deficits, species and additives (Table 3). As expected, significant differences existed between each saturation deficit. However, there were no significant differences between the TI_{50} values of the three nematodes tested. Between additives within each saturation deficit level, there was at least one significant difference. In an attempt to depict any similarities between additives within each saturation deficit level the mean ranks were calculated for each additive using the Kruskal-Wallis test (alpha = 0.05) (Table 4). Observation of these ranked values show similarities between several additives leading to a grouping effect as follows: Group 1 - distilled water; Group 2 - Soybean, Savol, Cottonseed, Sunspray and Citrus; Group 3 - Klearol, Pyronyl and Exxon; and Group 4 - Base, Heavy and 2251. However, the separation of the additives into these 4 groups is not always consistent at each saturation deficit level. For example, in Figure 7 using *S. bibionis*, Groups 1 to 3 converge at the 19.4 millibar saturation deficit level but remain separated from Group 4. As the saturation deficit decreases, Groups 3 and 4 converge and remain separate from Groups 1 and 2. These inconsistences also occur in Figures 8 and 9 where L1C and All strain are used,

Figure 7. The mean TI_{50} values of *S. bibionis* immersed in distilled water and additive droplets (conc. 10% additive) and exposed at saturation deficits of 19.4, 8.8 and 3.5 millibars for predetermined time intervals. (Note: Curves are fit by eye for illustrative purposes.)


3.5
8.8
19.4
Saturation Deficit (millibars)
1
2
3
4
40
80
120
160
200
240
280
320
TI 50 (minutes)

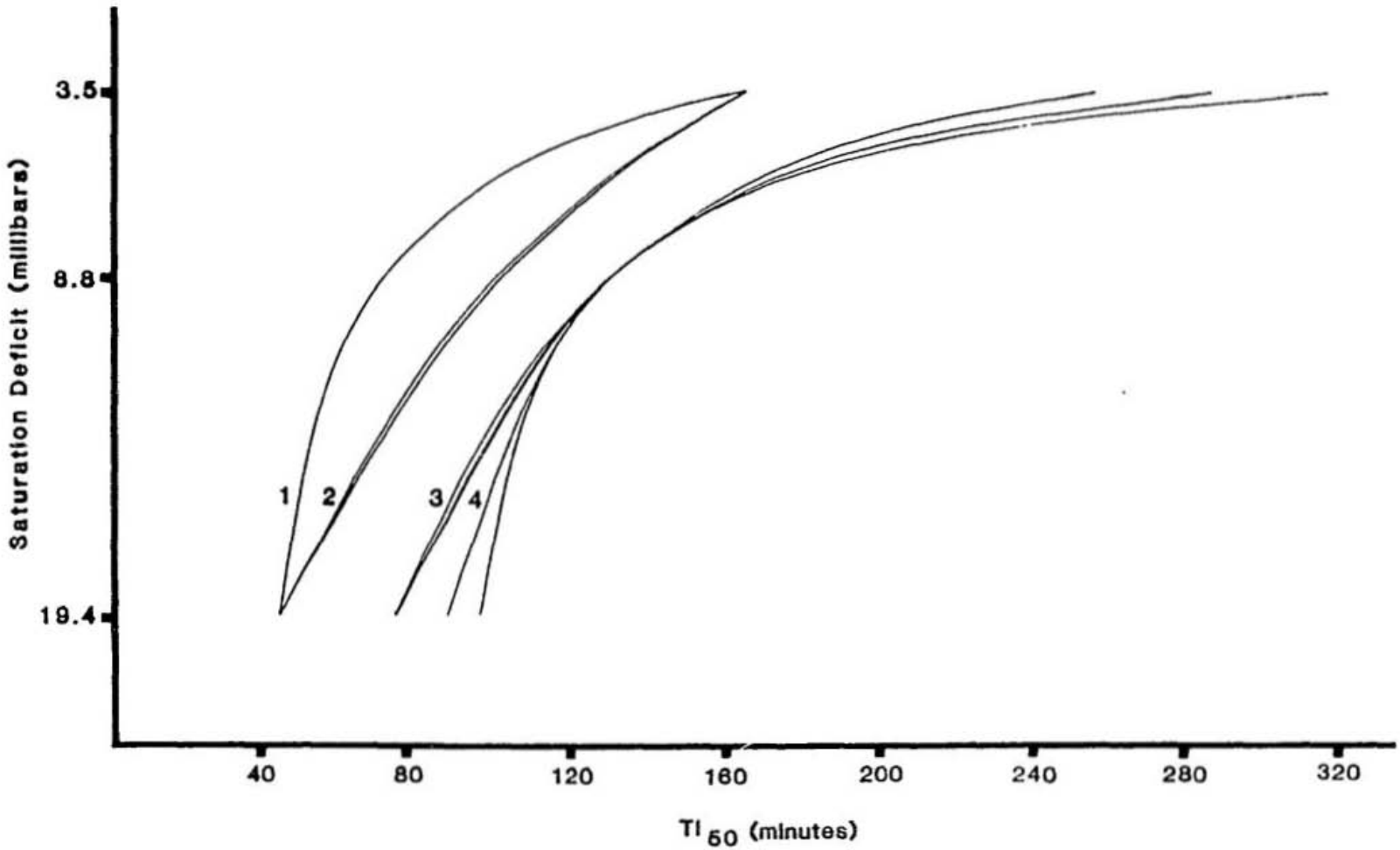

3.5
8.8
19.4
Saturation Deficit (millibars)
1
2
3
4
40
80
120
160
200
240
280
320
TI 50 (minutes)

Figure 8. The mean TI_{50} values of L1C immersed in distilled water and additive droplets (conc. 10% additive) and exposed at saturation deficits of 19.4, 8.8 and 3.5 millibars for predetermined time intervals. (Note: Curves are fit by eye for illustrative purposes.)

Figure 9. The mean TI_{50} values of All strain immersed in distilled water and additive droplets (conc. 10% additive) and exposed at saturation deficits of 19.4, 8.8 and 3.5 millibars for predetermined time intervals. (Note: Curves are fit by eye for illustrative purposes.)

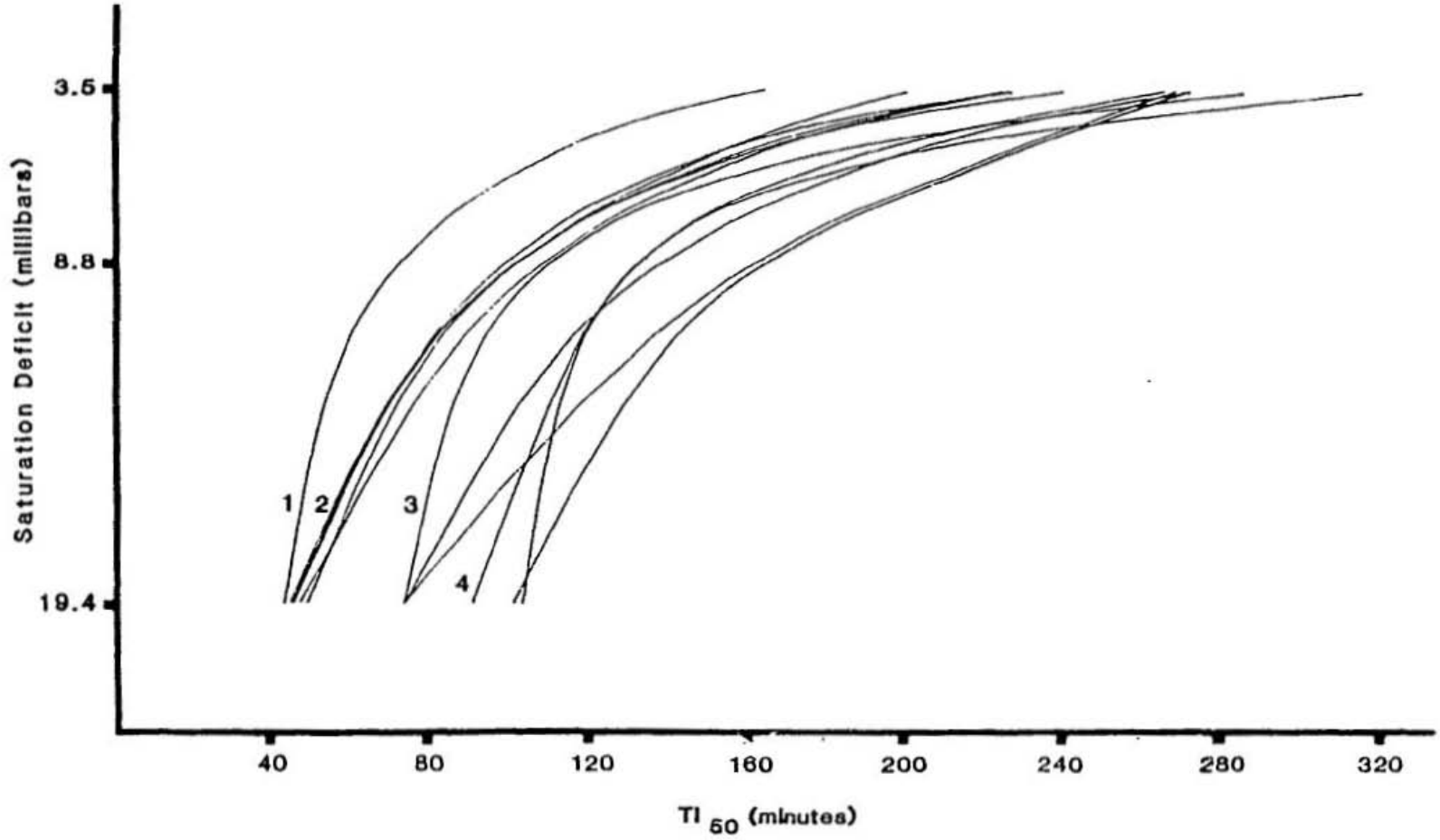

3.5
8.8
19.4
Saturation Deficit (millibars)
1
2
3
4
40
80
120
160
200
240
280
320
TI 50 (minutes)

Table 2. The mean TI_{50} values (in minutes), determined by probit analysis, of *S. bibionis*, L1C and All strain immersed in distilled water and additive droplets (conc. 10% additive) and exposed at saturation deficits of 19.4, 8.8 and 3.5 millibars for predetermined time intervals.[a,b]

	Saturation Deficit																	
	19.4						8.8						3.5					
	S. bibionis		L1C		All strain		*S. bibionis*		L1C		All Strain		*S. bibionis*		L1C		All Strain	
ADDITIVE	n	TI50	n	TI50	n	TI50	n	TI50	n	TI50	n	TI50	n	TI50	n	TI50	n	TI50
Distilled Water	165	42.4	213	42.4	201	42.4	152	73.5	336	73.5	247	73.5	122	164.3	204	164.3	186	164.3
Soybean	109	31.3	229	42.4	389	57.3	141	103.9	393	103.9	241	103.9	270	149.6	255	164.3	220	224.5
Savol	139	31.7	283	42.4	306	56.5	171	103.9	421	103.9	301	117.6	251	149.8	257	164.3	239	234.5
Cottonseed	132	31.6	281	42.4	248	54.9	180	103.9	391	103.9	278	103.9	264	149.3	211	164.3	242	224.5
Sunspray	141	42.4	263	42.4	255	54.1	147	103.9	377	103.9	519	103.9	256	150.0	242	164.3	298	207.2
Citrus	165	42.4	219	42.4	316	55.5	225	110.2	391	103.9	276	93.6	243	147.8	231	164.3	248	234.4
Klearol	212	57.5	282	73.5	262	73.5	145	134.1	406	134.2	297	146.5	304	254.6	225	254.6	243	270.2
Pyronyl	227	55.5	222	73.5	241	73.5	229	137.8	434	134.2	260	118.0	259	242.3	266	254.6	180	284.6
Exxon	130	42.4	219	73.5	390	73.5	160	134.2	429	134.2	243	164.3	196	254.6	363	284.6	261	270.8
Base	276	73.5	236	73.5	379	96.5	288	148.0	465	134.2	238	172.4	275	233.3	411	314.6	247	270.6
Heavy	156	73.5	295	89.6	312	103.9	139	134.2	431	134.2	219	134.2	281	277.5	407	314.6	246	314.6
2251	171	73.5	237	91.5	307	90.3	155	146.2	311	134.2	265	134.2	259	242.4	387	314.6	195	314.6

* The confidence limits for each mean TI50 value are shown in Appendices Y1, Y2 and Y3.

** n is the total number of nematodes contained in 10 replicates.

Table 3. Kuskal-Wallis test showing significant differences (indicated by *) between saturation deficits, species and additives (alpha = 0.01).

FACTOR	TEST RESULT
Between saturation deficit levels	
*19.4 vs 8.8 millibars	$X^2 = 48.12$, d.f. = 1, $p<0.01$
*19.4 vs 3.5 millibars	$X^2 = 49.07$, d.f. = 1, $p<0.01$
*3.5 vs 8.8 millibars	$X^2 = 46.00$, d.f. = 1, $p<0.01$
Between species	
S. bibionis vs L1C	$X^2 = 0.27$, d.f. = 1, $p>0.61$
S. bibionis vs All strain	$X^2 = 0.97$, d.f. = 1, $p>0.33$
L1C vs All strain	$X^2 = 0.34$, d.f. = 1, $p>0.56$
Between additives within each saturation deficit level	
*19.4 millibars	$X^2 = 26.58$, d.f. = 1, $p<0.01$
*8.8 millibars	$X^2 = 30.87$, d.f. = 1, $p<0.01$
*3.5 millibars	$X^2 = 27.39$, d.f. = 1, $p<0.01$

Table 4. The mean rank for each additive within each group under each saturation deficit level using the Kruskal-Wallis test procedure (alpha = 0.05).

Group	Additive	Saturation Deficit		
		19.4	8.8	3.5
1	Distilled Water	9.00	2.00	9.50
	Soybean	10.00	10.50	9.33
	Savol	10.33	13.00	10.83
2	Cottonseed	9.00	10.50	9.00
	Sunspray	11.00	10.50	9.50
	Citrus	11.83	10.50	9.50
	Klearol	24.67	26.17	24.33
3	Pyronyl	23.50	25.17	24.67
	Exxon	20.67	28.67	27.33
	Base	29.33	31.80	26.00
4	Heavy	31.50	25.50	32.33
	2251	31.17	27.67	29.67

respectively.

The additives contained within each group showed no significant differences with regard to their TI_{50} value when tested at each saturation deficit level (Table 5). Each of the groups tested in Table 5 showed significant differences from each other at each saturation level except for Groups 3 and 4 at both the lowest (3.5 millibars) and median (8.8 millibars) saturation deficit levels and Groups 1 and 2 at the highest (19.4 millibars) and the lowest (3.5 millibars) saturation deficit levels.

It is also apparent that the additives of the first group give the least enhancement; those of the second group give a median enhancement; while the additives of the third group give the best protection. This trend is somewhat more evident from the curves in Figures 7, 8 and 9. Similar to the responses in Part I of this study, there is an apparent levelling off of the curves between the 8.8 and 3.5 millibar levels indicating that as the saturation deficit decreased, from 8.8 to 3.5 millibars, the nematodes showed an increased tolerance to dessication, indicating a possible threshold saturation level.

PART III. Viscosity Measurements

Figure 10 depicts the relative viscosities of distilled water and the remaining additives. Statistical analysis using Scheffe's test (alpha = 0.01) indicated that several additives were similar according to their relative viscosities. The materials grouped together based on their similar viscosities were as follows: Group 1 - Soybean; Group 2 - Savol; Group - 3 Cottonseed; Group 4 - Sunspray and Citrus; Group 5 - Exxon, Klearol and Pyronyl; and Group 6 - Base, Heavy and 2251. Group 1 had the highest viscosity while Group 6 had the lowest viscosity. Appendix X outlines the mean relative viscosity and standard error for each of the additives tested.

Table 5. Kruskal-Wallis test results showing significant differences (indicated by *) between the additives within each group and the groups of additives (alpha = 0.05).

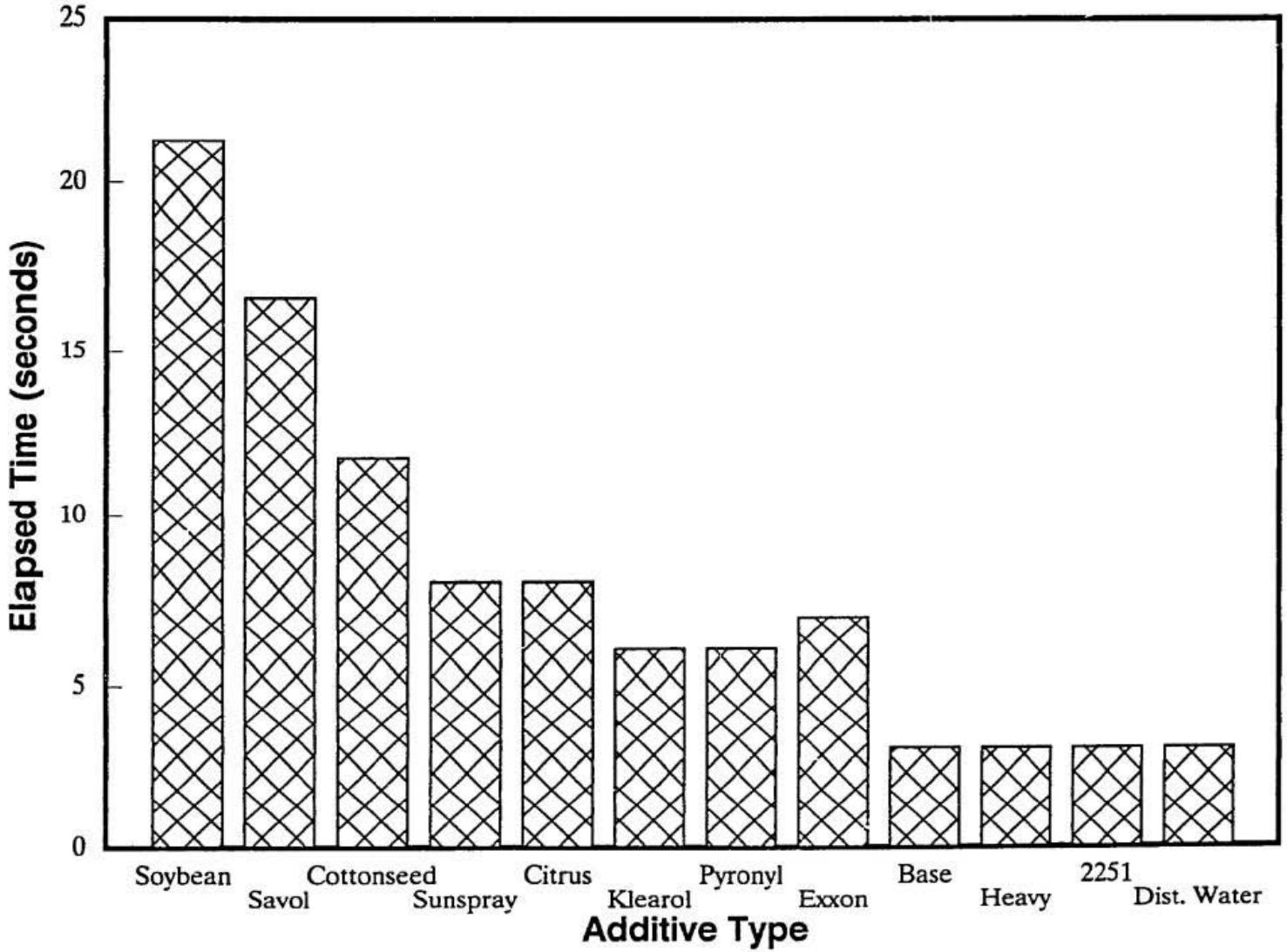
FACTOR	TEST STATISTIC RESULT
Within Groups	
19.4 millibars	
Additives 2-6 (Group 2)	$X^2 = 0.37$, d.f.=4, p>0.99
Additives 7-9 (Group 3)	$X^2 = 0.12$, d.f.=2, p>0.94
Additives 10-12 (Group 4)	$X^2 = 0.31$, d.f.=2, p>0.85
8.8 millibars	
Additives 2-6 (Group 2)	$X^2 = 1.53$, d.f.=4, p>0.35
Additives 7-9 (Group 3)	$X^2 = 0.76$, d.f.=2, p>0.69
Additives 10-12 (Group 4)	$X^2 = 3.23$, d.f.=2, p>0.19
3.5 millibars	
Additives 2-6 (Group 2)	$X^2 = 0.21$, d.f.=4, p>0.99
Additives 7-9 (Group 3)	$X^2 = 1.17$, d.f.=2, p>0.56
Additives 10-12 (Group 4)	$X^2 = 1.39$, d.f.=2, p>0.50
Between Groups	
19.4 millibars	
Group 1 vs 2	$X^2 = 0.15$, d.f.=1, P>0.69
*Group 2 vs 3	$X^2 = 11.97$, d.f.=1, P<0.01
*Group 3 vs 4	$X^2 = 7.63$, d.f.=1, P<0.01
*Group 3 vs 1	$X^2 = 5.84$, d.f.=1, p<0.02
*Group 4 vs 1	$X^2 = 6.55$, d.f.=1, P<0.01
*Group 4 vs 2	$X^2 = 16.78$, d.f.=1, p<0.01
8.8 millibars	
*Group 1 vs 2	$X^2 = 10.14$, d.f.=1, p<0.01
*Group 2 vs 3	$X^2 = 18.59$, d.f.=1, p<0.01
Group 3 vs 4	$X^2 = 0.53$, d.f.=1, p>0.47
*Group 3 vs 1	$X^2 = 6.55$, d.f.=1, p<0.01
*Group 4 vs 1	$X^2 = 7.21$, d.f.=1, p<0.01
*Group 4 vs 2	$X^2 = 18.83$, d.f.=1, p<0.01

Table 5 (cont'd)

3.5 millibars

Group 1 vs 2	X^2 = 0.00, d.f.= 1, p=1.00
*Group 2 vs 3	X^2 =16.42, d.f.= 1, p<0.01
Group 3 vs 4	X^2 = 2.75, d.f.= 1, p>0.17
*Group 3 vs 1	X^2 = 6.58, d.f.= 1, p<0.01
*Group 4 vs 1	X^2 = 6.80, d.f.= 1, p<0.01
*Group 4 vs 2	X^2 =15.53, d.f.= 1, p<0.01

Figure 10. The mean elapsed time for 9mls of each additive and distilled water to dispense from a 10ml pipette at a temperature of 23°C.


25
20
15
10
5
0
Elapsed Time (seconds)
Soybean
Savol
Cottonseed
Sunspray
Citrus
Klearol
Pyronyl
Exxon
Base
Heavy
2251
Dist. Water
Additive Type

DISCUSSION

Differences were observed between the nematodes with regard to their ability to resist desiccation. The results indicate that *S. bibionis*, *Steinernema* sp. Nfld. strain No. 1 (L1C strain) and *Steinernema feltiae* (All strain) had the lowest, median and highest resistance to desiccation, respectively. Differences between several species of nematodes to resist desiccation under various humidity levels have been demonstrated by a number of workers. Demeure, *et al.* (1979), observed differences between four species of nematodes with regard to their ability to resist desiccation. It was demonstrated that the desert nematode, *Acrobeloides* sp. Thorne, a bacterial feeder, was more resistant to desiccation than *Aphelenchus avenue* Bastian, a fungal feeder, *Helicotylenchus dihystera* Sher, a plant parasitic nematode and *Scutellonema brachyurum* Andrassy, a plant parasitic nematode. Under various humidity regimes, Perry (1977a,c) found *Ditylenchus dipsaci* (Kuhn) Filipjev to be superior in resisting desiccation than *D. myceliophagus*. In an experiment by Simons (1973), *Tylenchorhynchus dubius* exhibited a superior ability over *Rotylenchus robustus* to survive for a longer period of time while exposed at a 93% relative humidity level. Significant differences were also detected between the different larval stages of nematodes to resist desiccation. In the report by Perry (1977b), the fourth stage larvae of *D. dipsaci* exhibited more tolerance to desiccation than either the third or adult stages. These experiments have demonstrated that variations can arise between species, life stages and habitat.

The results of this part of the study clearly indicate that significant differences in desiccation tolerance can exist between entomogenous nematodes. These findings have particular relevance to the efficacy of these species with regard to their potential as biocontrol agents. Past research has also indicated the importance of available moisture during the post-spray period to increase the probability of infection by the nematode. The results of this study indicate that the All strain has the greatest tolerance to desiccation

and thus has the best potential as a biocontrol agent.

Although not investigated directly in this study, extensive research has indicated that the rate of water loss during dehydration is a vital factor in the ability of nematodes to withstand desiccation (Demeure, *et al.* 1979; Perry, 1977a,b,c; Simons, 1973; Womersley, 1978, 1980). Although attempts to quantify "slow drying" or "fast drying" has only met with conjecture, many studies have pointed out that survival and hence revival in nematodes is higher when exposed to slow drying than when exposed to fast drying environments. This phenomenon, termed anhydrobiosis, has been reviewed by Crowe (1971). Simons and Poinar (1973) demonstrated the importance of gradual dehydration in the survival of nematodes whereby more than 90% of the infectives of *Steinernema carpocapsae* were able to survive for more than 12 days when dried over a series of moisture regimes from 96.0% relative humidity decreasing gradually to 10% relative humidity. Crowe and Madin (1974, 1975) emphasized the importance of slow drying in order for the nematode to prepare for extensive water loss under extreme saturation deficits.

One may speculate on the effects of anhydrobiosis as it relates to the data collected in Part A of this study. There is a similar response by each nematode, as depicted by each curve, whereby a near vertical response occurs up to the 6.2 millibar level. After this level, the slope of the curves decrease toward a horizontal position. The vertical portion of the curves may represent a fast drying response whereby the nematodes are undergoing minimal adaptational response to the rapid loss of water. When the 6.2 millibar level is reached, the rate of water loss is decreased allowing the nematodes more time to envoke an adaptational response. Hence, this adaptational response allows the nematodes to remain mobile for longer period of time. Several reports have indicated that differential drying is evident when nematodes are dried in clumps or aggregates as opposed to being dried individually (Kamionek *et al.* 1974; Ellenby, 1968; Babatola, 1980; Crowe and Madin, 1974, 1975). In this study, the number of nematodes per

exposure was low, therefore, no clumping was observed.

The results of the desiccation tolerance experiment and the viscosity test indicate an inverse relationship between the TI_{50} values and the relative viscosities of the additives. Additives which provided the best, median and lowest (Groups 4, 3 and 2, respectively) had the lowest, median and highest relative viscosities (Groups 2, 3 and 4, respectively).

It was observed throughout this experiment that some of the additives, when mixed with water, did not form a distinct droplet shape but rather caused the water to spread in a fan shape pattern with the remaining oil content floating on a small portion of the surface of the water. This occurred frequently with Soybean, Savol, Cottonseed, Sunspray and Citrus additives (Group 1) which provided the least protection of the nematodes contained within the droplets. It can only be speculated, therefore, that the relative viscosity may be related to the ability of an additive to form a distinct droplet shape which in turn may have an effect on the rate of evaporation of the water contained in it. If the droplets conform to this fan shape, then the increased surface area exposed to the air may result in a higher rate of evaporation of the water, hence, decreasing the length of time in which the nematodes can remain mobile. The remaining additives had a tendency to form distinct water droplets which may have increased the evaporation time of the water component.

A report by Bedding (1976) pointed out that the anti-evaporative quality of various additives may not be the only factor which may determine a superior additive. The oxygen holding capacity of an additive may also be a limiting factor which may affect the longevity of nematodes immersed in droplets. He further elaborated by pointing out that nematodes can survive in deep oil without water for several months but rapidly desiccate when placed in small oil droplets. However, the addition of 5% paraffin wax to the oil droplets prevented the desiccation of the nematodes for several days. This response was similar to several preliminary experiments done by the investigator of this

study whereby the addition of 8% paraffin wax to mineral and paraffin oils extended nematode mobility for up to 4 to 5 days under extreme drying conditions. Observation of these droplets indicated the superior ability of paraffin wax to extend nematode mobility. Therefore, it seems logical to assume that the nematodes were able to remain mobile for a longer period of time due to the reduction of oxygen diffusion from the droplet of oil. However, although the paraffin wax may have reduced oxygen diffusion, the oxygen consumption by the nematode during this time has to be considered.

In a similar report by Shapiro, *et al.* (1985), the same additives were evaluated with regard to their antidesiccant ability. Their findings varied with those of this report and were probably due to the different methodologies employed in each study. Their findings were based on indirect measurements such as host mortality, nematode development and fecundity. In addition, their exposures were not conducted in droplet form and in many cases did not allow for clumping by using very low numbers of nematodes per exposure. Hence, these factors may have contributed to the differences between the findings of both studies.

One may only speculate on why there were no significant differences between the species in Part B of the study. Through direct observation of the droplets containing water, additive and nematodes, it was evident that the additives were not soluble in water and their distinct separation was easily detected using a dissecting microscope. It was also observed that once the water component of the droplet was totally evaporated, nematode immobility was almost immediate. Therefore, it would be safe to assume that the additive served only to protect the water component of the droplet and did not provide any protection of the nematode in its pure state due to possible toxic effects causing nematode immobility. Direct toxicity of these additives on these nematode species is not known.

Results of this and other studies indicate the difficulty in finding a suitable and effective method of enhancing nematode mobility and survival during the post-spray period against foliar feeding insect pests. The differences between these additives with

regard to their antidesiccant ability may be related to their physical properties, such as viscosity, as well as their chemical properties. In addition to the laboratory analysis of this study, further evaluation of their response to varying temperatures and ultra-violet radiation levels would be useful. Although, some additives were more effective antidesiccants than others, they deserve to be tested under more vigorous field conditions. One hopes that all the information gathered on these tests may be used to design unique and innovative methods of protecting the nematodes during the post-spray period and thus increase their effectiveness as biocontrol agents. Recently, Kaya and Nelson (1985) found that infectives of *S. feltiae* and *H. heliothidis* survived for eight months when encapsulated inside calcium alginate pellets. Similarly, Poinar, *et al.* (1985) found infectives of *Neoaplectana carpocapsae* alive in sodium alginate capsules after 7 days when exposed to varied moisture levels. Although these studies show promise in significantly extending nematode survival, they are useful only in soil applications and are not conducive to foliar applications.

SUMMARY AND CONCLUSIONS

Previous studies have clearly indicated the potential of entomophilic nematodes as biocontrol agents for many insect pests. Many of the field trials up to the present time have yielded various degrees of success indicating the complexities in using nematodes as biocontrol agents. Although this study did not attempt field trials, the information gathered would be helpful in future studies which attempt to evaluate the potential of certain species of nematodes as biological control agents and the efficacy of various spray additives as potential antidesiccants.

1. Differences exist between various species with regard to their tolerance to desiccation. Laboratory analysis in this study has shown All strain to be superior compared to *S. bibionis* or L1C strain. Any species of nematodes should be evaluated not only with regard to their desiccation tolerance but also temperature tolerance, mobility, pathogenicity, fecundity and ultra-violet radiation tolerance.

2. The saturation deficit level during both the spray and post-spray periods is of prime importance to provide adequate moisture level to ensure nematode mobility. Specifically, the rate of change in these saturation levels is crucial to the mobility of nematodes used in a spray program. A slow rate of change may allow the nematodes to adapt to the loss of water and prevent irreversible cellular damage. A fast rate would not allow this adaptational process to occur resulting in certain death of the nematodes. Therefore, conducting spray operations during periods of low humidity levels could cause significant reduction in the effectiveness of the spray regime by causing rapid nematode desiccation.

3. The type of host habitat should be carefully assessed to determine whether the host is accessible to nematode infection and also whether the habitat will provide adequate

moisture levels to ensure nematode survival during the post spray period. It is possible that in cryptic habitats such as soil where fluctuations in moisture levels are gradual, nematode protection may enhanced and therefore be more effective in reducing populations of insect pests.

4. Laboratory analysis of potential antidesiccants should be conducted under controlled saturation levels. The data collected should accurately reflect the ability of the additives to provide adequate protection of the infective stage of the nematodes against against desiccation under various saturation levels. It is important to use low numbers of nematodes per droplet to prevent clumping and therefore obtain a more accurate measure of the effectiveness of the additive. It can only be speculated from this study that the ability of an additive to conform to distinct droplet shape may be related to its viscosity which, in turn, may affect its ability to additive to prevent water evaporation. Other properties such as toxicity must be investigated prior to field tests.

Results of this and other studies indicate the difficulty in finding a suitable and effective method of enhancing nematode mobility and survival during the post spray period. Recently, Kaya and Nelson (1985) found that infectives of *S. feltiae* and *H. heliothidis* survived for 8 months when encapsulated inside calcium alginate pellets. Similiarly, Poinar *et al.* (1985) found infectives of *N. carpocapsae* to be living in sodium alginate capsules after 7 days when subjected to various substrates having varied moisture levels. Although, these methods show promise, they are only useful in soil applications.

These areas of research have shown progress in attempts to overcome nematode desiccation in spray regimes by encapsulating the nematodes. If successful, new avenues of research may develop whereby the use of baits, scents and traps will make these methods more effective in controlling major insect pests.

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APPENDICES

APPENDIX A

Compilation from Landolt-Borstein vapor pressures and specific gravities of glycerin-water solutions of various percentages at 20°C.

Glycerol Solution (%)	Saturation Deficit (millibars)	Specific Gravity	Relative Humidity (%)
0	0.000	0.098	100.0
15.5	0.701	1.036	97.0
25.0	1.683	1.059	92.8
35.0	3.459	1.085	85.2
50.0	6.217	1.126	73.4
60.0	8.787	1.153	62.4
75.0	13.118	1.194	44.3
85.0	15.472	1.215	33.8
92.0	19.421	1.240	16.9
98.0	22.974	1.256	1.7
100.0	23.371	1.261	0.0

APPENDIX B

Mean percent immobility of *S. bibionis* exposed at various saturation deficit for predetermined time intervals.

Exposure Time (minutes)	Saturation Deficit (millibars)						
	23.4	19.4	15.5	13.0	8.8	6.2	3.5
2	0 (0)						
4	2 (0)						
6	94 (2)						
8	100 (0)						
10		0 (0)	0 (0)				
15		100 (0)	4 (2)	0 (0)			
20			78 (6)	34 (4)			
25			100 (0)	62 (5)	0 (0)		
30				94 (2)	14 (0)	0 (0)	
35				100 (0)	67 (2)		
40					86 (2)	32 (4)	
45					100 (0)		
50						88 (4)	0 (0)
55							
60						100 (0)	24 (6)
70							64 (2)
80							96 (2)
90							100 (0)

* Standard error is shown in parentheses after each mean.

APPENDIX C

Mean percent immobility of L1C strain exposed at various saturation deficits for predetermined time intervals.

Exposure Time (minutes)	Saturation Deficit (millibars)						
	23.4	19.4	15.5	13.0	8.8	6.2	3.5
10	0 (0)						
20	26 (7)	0 (0)	0 (0)				
30	100 (0)	34 (9)	26 (2)	0 (0)			
40		90 (3)	50 (5)	32 (2)	0 (0)		
50		100 (0)	88 (4)	76 (2)	28 (2)	0 (0)	
60			100 (0)	100 (0)	86 (2)	40 (4)	
70					96 (2)	86 (5)	
80					100 (0)	100 (0)	
90							
100							0 (0)
110							4 (2)
120							10 (3)
130							68 (4)
140							100 (0)

* Standard error is shown in parentheses after each mean.

APPENDIX D

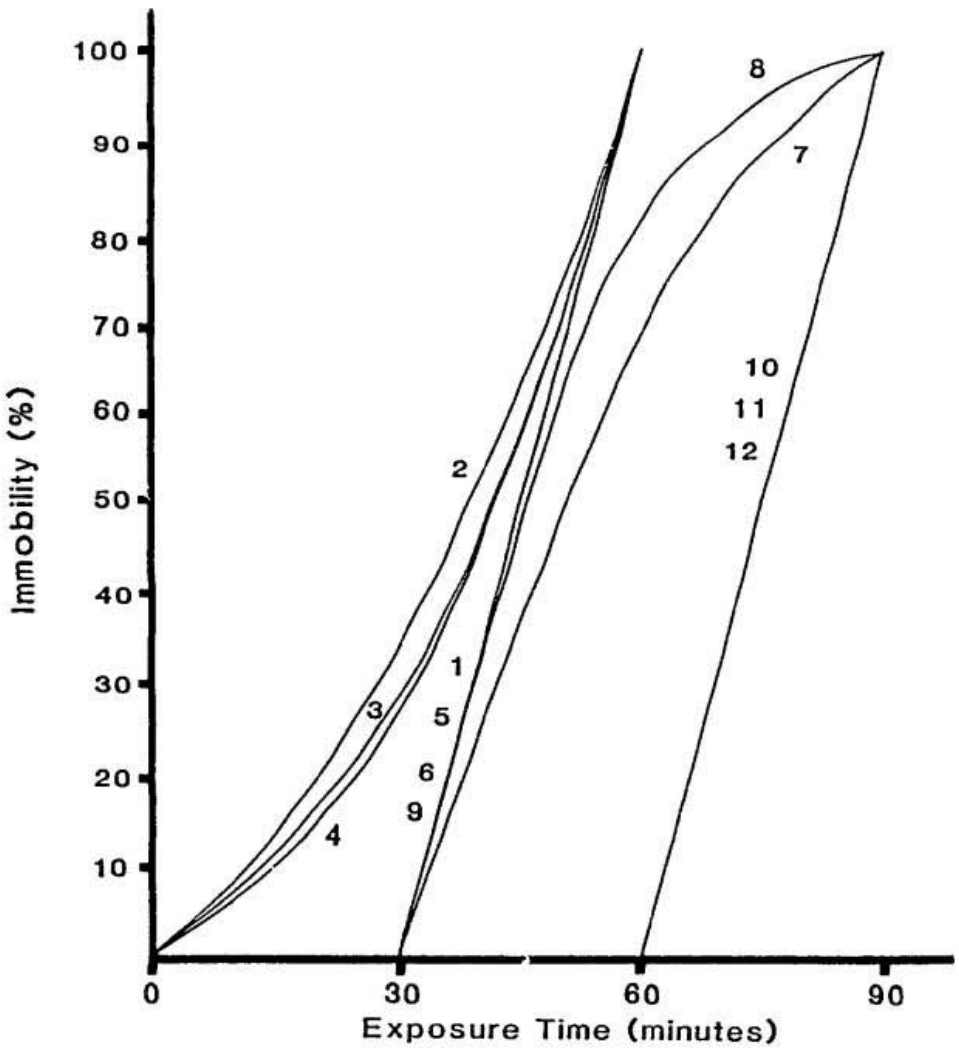
Mean percent immobility of All strain exposed at various saturation deficits for predetermined time intervals.

Exposure Time (minutes)	Saturation Deficit (millibars)						
	23.4	19.4	15.5	13.0	8.8	6.2	3.5
10							
20	0 (0)						
30	76 (7)	0 (0)					
40	100 (0)	48 (12)	0 (0)				
50		82 (7)	44 (4)	0 (0)			
60		100 (0)	74 (6)	44 (6)			
70			94 (3)	68 (2)	0 (0)		
80			96 (4)	82 (2)	26 (2)	0 (0)	
90			100 (0)	94 (3)	46 (4)	24 (2)	
100				100 (0)	84 (2)	69 (4)	
110					94 (3)	79 (3)	
120					100 (0)	90 (1)	
130						100 (0)	
140							
150							0 (0)
160							6 (2)
170							14 (2)
180							30 (4)
190							38 (4)
200							48 (4)
210							64 (4)
220							74 (2)
230							86 (2)
240							94 (2)
250							98 (2)
260							98 (2)
270							100 (0)

* Standard error is shown in parentheses after each mean.

APPENDIX E

The mean percent immobility of *S. bibionis* immersed in distilled water and additive droplets (conc. 10% additive) and exposed at a saturation deficit of 19.4 millibars for predetermined time intervals. (1 - Distilled water, 2 - Soybean, 3 - Savol, 4 - Cottonseed, 5 - Sunspray, 6 - Citrus, 7 - Klearol, 8 - Pyronyl, 9 - Exxon, 10 - Base, 11 - Heavy, 12 - 2251. Curves are fit by eye for illustrative purposes.)


Immobility (%)
100
90
80
70
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40
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90
Exposure Time (minutes)
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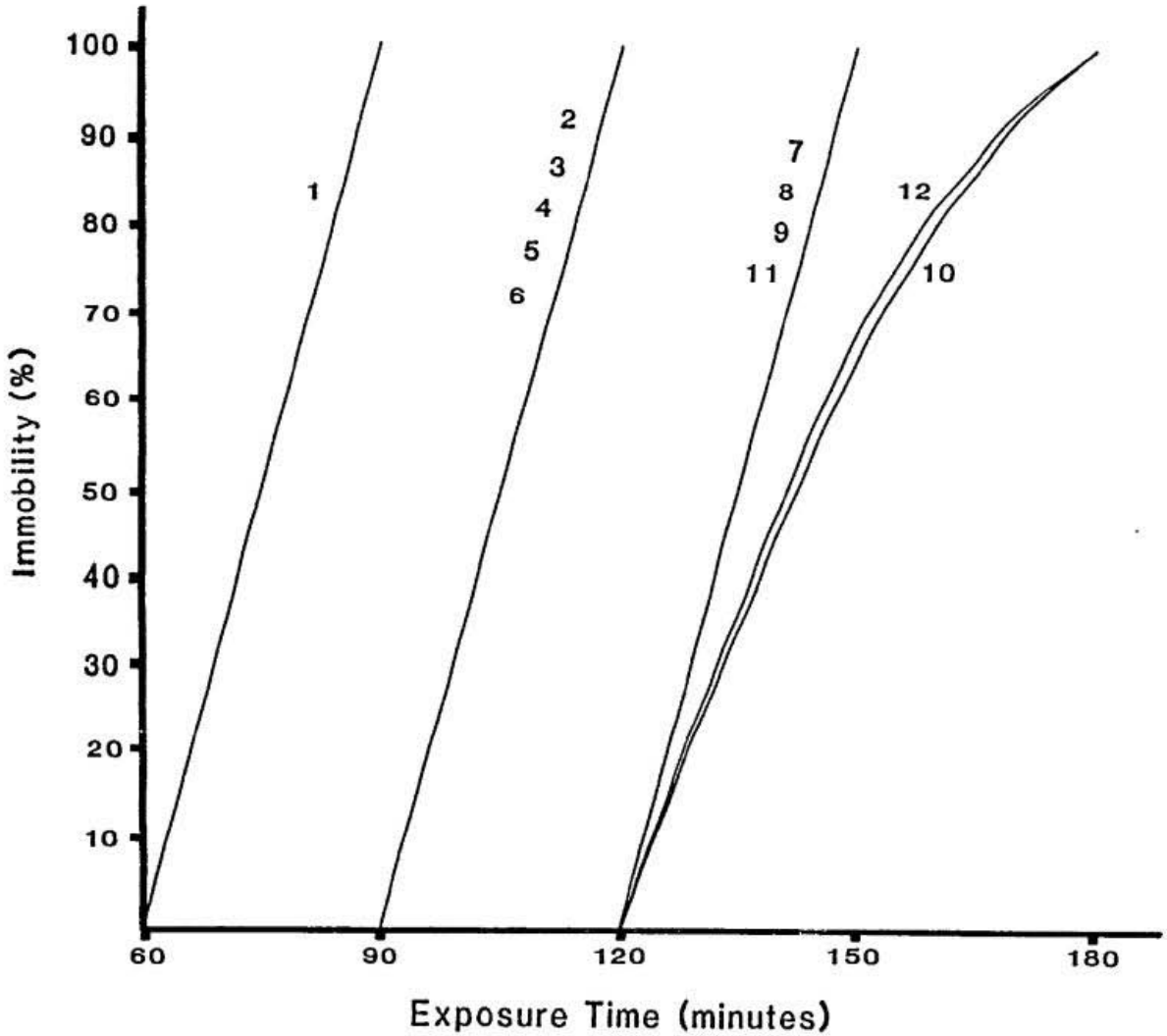
APPENDIX F

The mean percent immobility of *S. bibionis* immersed in distilled water and additive droplets (conc. 10% additive) and exposed at a saturation deficit of 8.8 millibars for predetermined time intervals. (1 - Distilled water, 2 - Soybean, 3 - Savol, 4 - Cottonseed, 5 - Sunspray, 6 - Citrus, 7 - Klearol, 8 - Pyronyl, 9 - Exxon, 10 - Base, 11 - Heavy, 12 - 2251. Curves are fit by eye for illustrative purposes.)


Immobility (%)
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Exposure Time (minutes)
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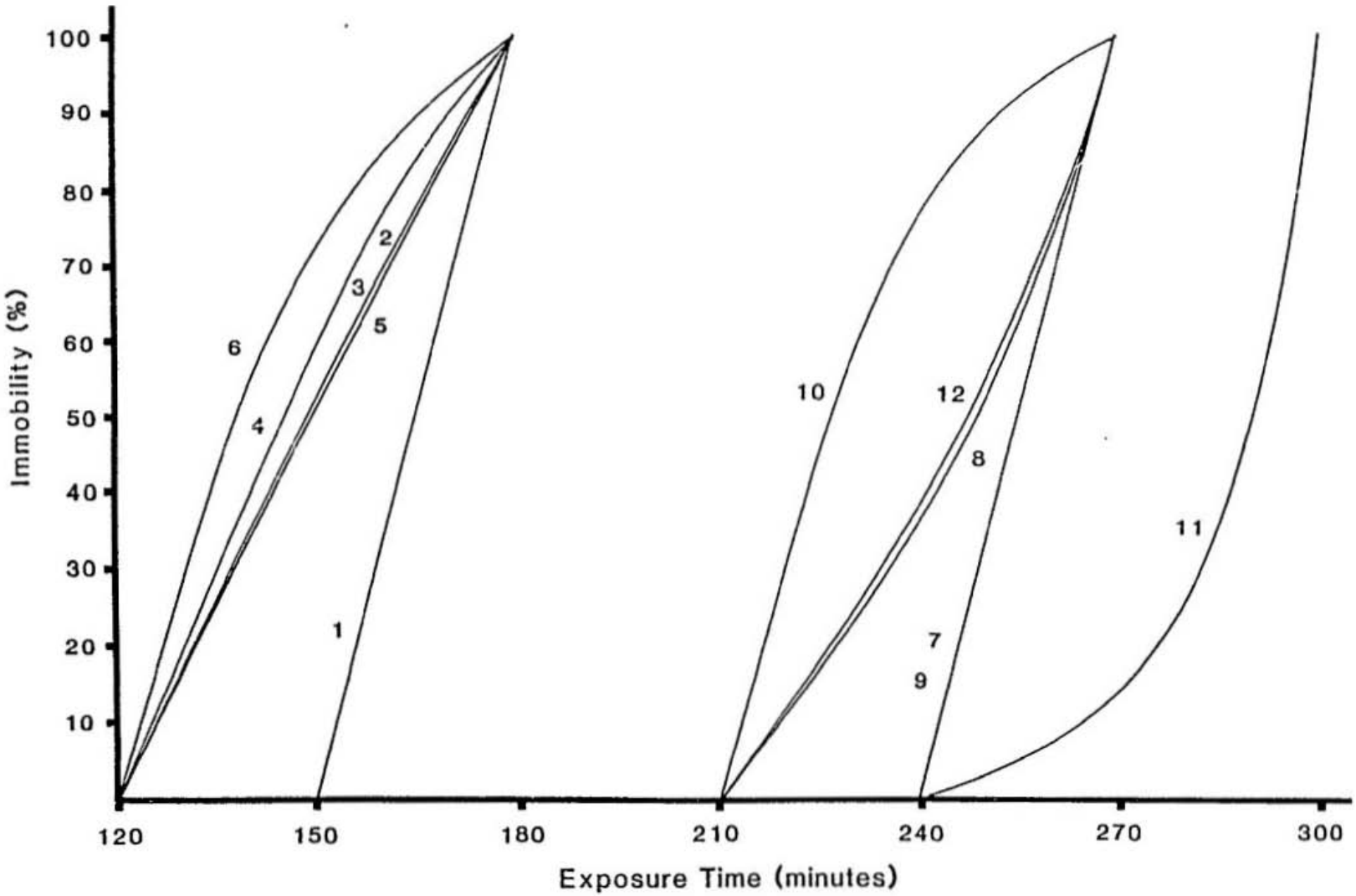
APPENDIX G

The mean percent immobility of *S. bibionis* immersed in distilled water and additive droplets (conc. 10% additive) and exposed at a saturation deficit of 3.5 millibars for predetermined time intervals. (1 - Distilled water, 2 - Soybean, 3 - Savol, 4 - Cottonseed, 5 - Sunspray, 6 - Citrus, 7 - Klearol, 8 - Pyronyl, 9 - Exxon, 10 - Base, 11 - Heavy, 12 - 2251. Curves are fit by eye for illustrative purposes.)


Immobility (%)
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Exposure Time (minutes)
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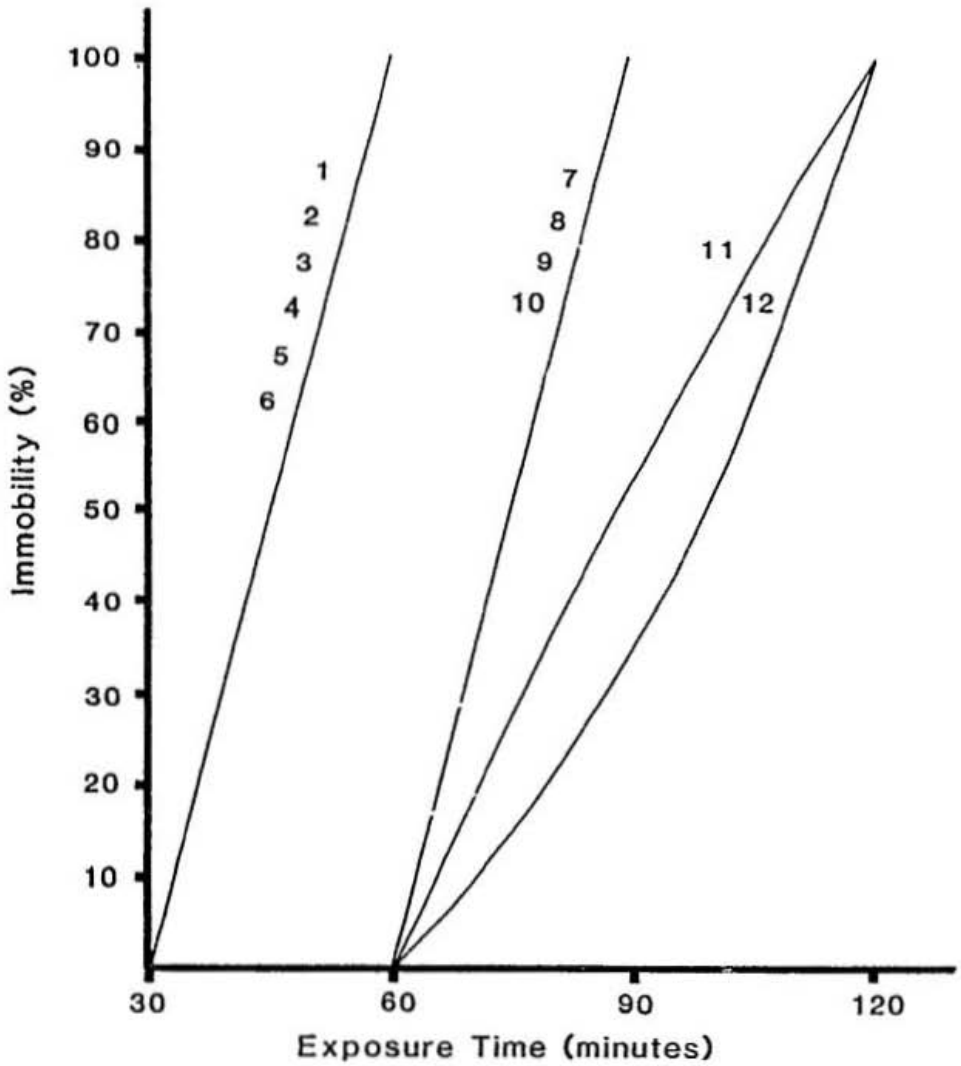
APPENDIX II

The mean percent immobility of L1C immersed in distilled water and additive droplets (conc. 10% additive) and exposed at a saturation deficit of 19.4 millibars for predetermined time intervals. (1 - Distilled water, 2 - Soybean, 3 - Savol, 4 - Cottonseed, 5 - Sunspray, 6 - Citrus, 7 - Klearol, 8 - Pyronyl, 9 - Exxon, 10 - Base, 11 - Heavy, 12 - 2251. Curves are fit by eye for illustrative purposes.)


Immobility (%)
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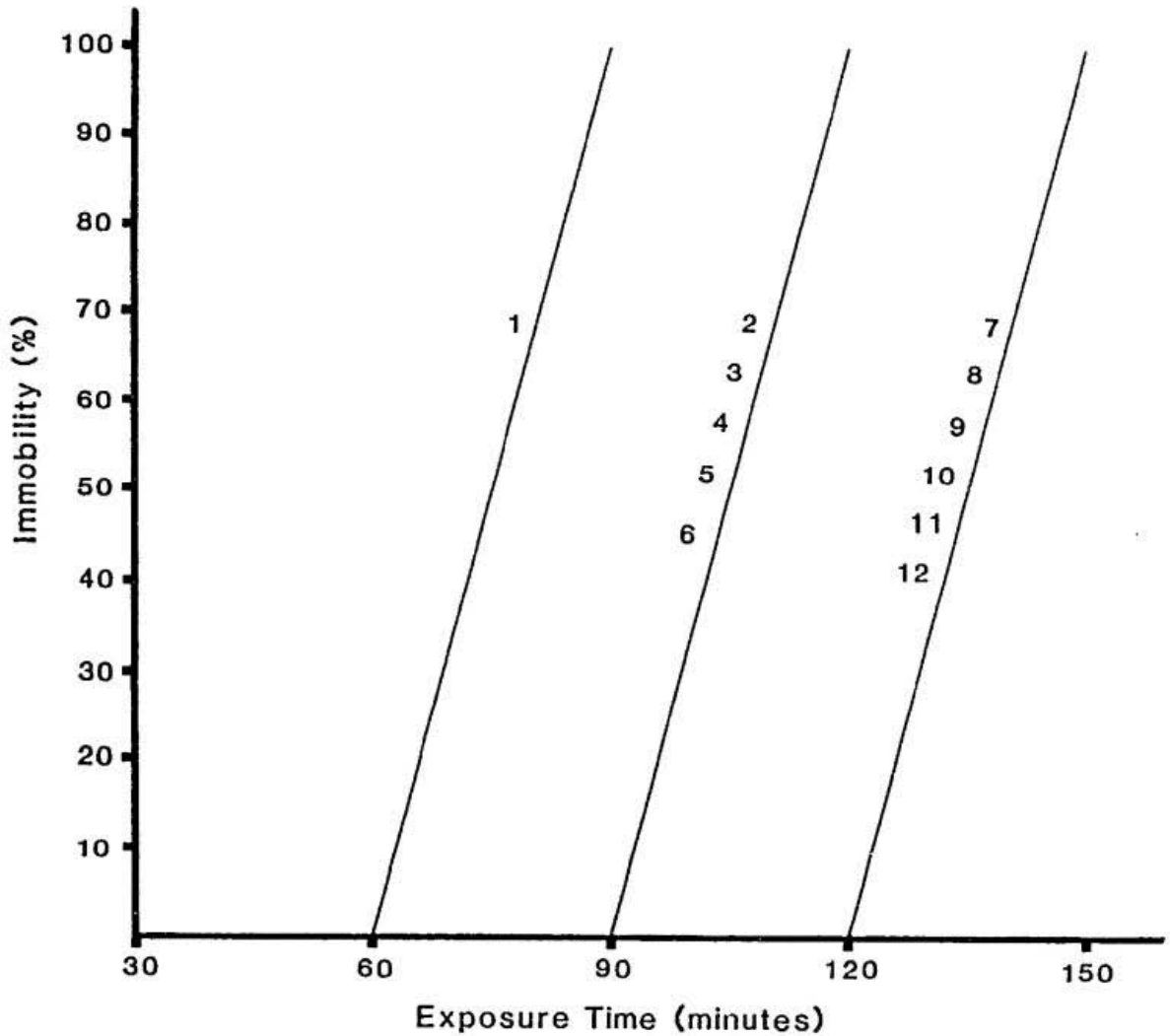
Exposure Time (minutes)

APPENDIX I

The mean percent immobility of L1C immersed in distilled water and additive droplets (conc. 10% additive) and exposed at a saturation deficit of 8.8 millibars for predetermined time intervals. (1 - Distilled water, 2 - Soybean, 3 - Savol, 4 - Cottonseed, 5 - Sunspray, 6 - Citrus, 7 - Klearol, 8 - Pyronyl, 9 - Exxon, 10 - Base, 11 - Heavy, 12 - 2251. Curves are fit by eye for illustrative purposes.)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Immobility (%)
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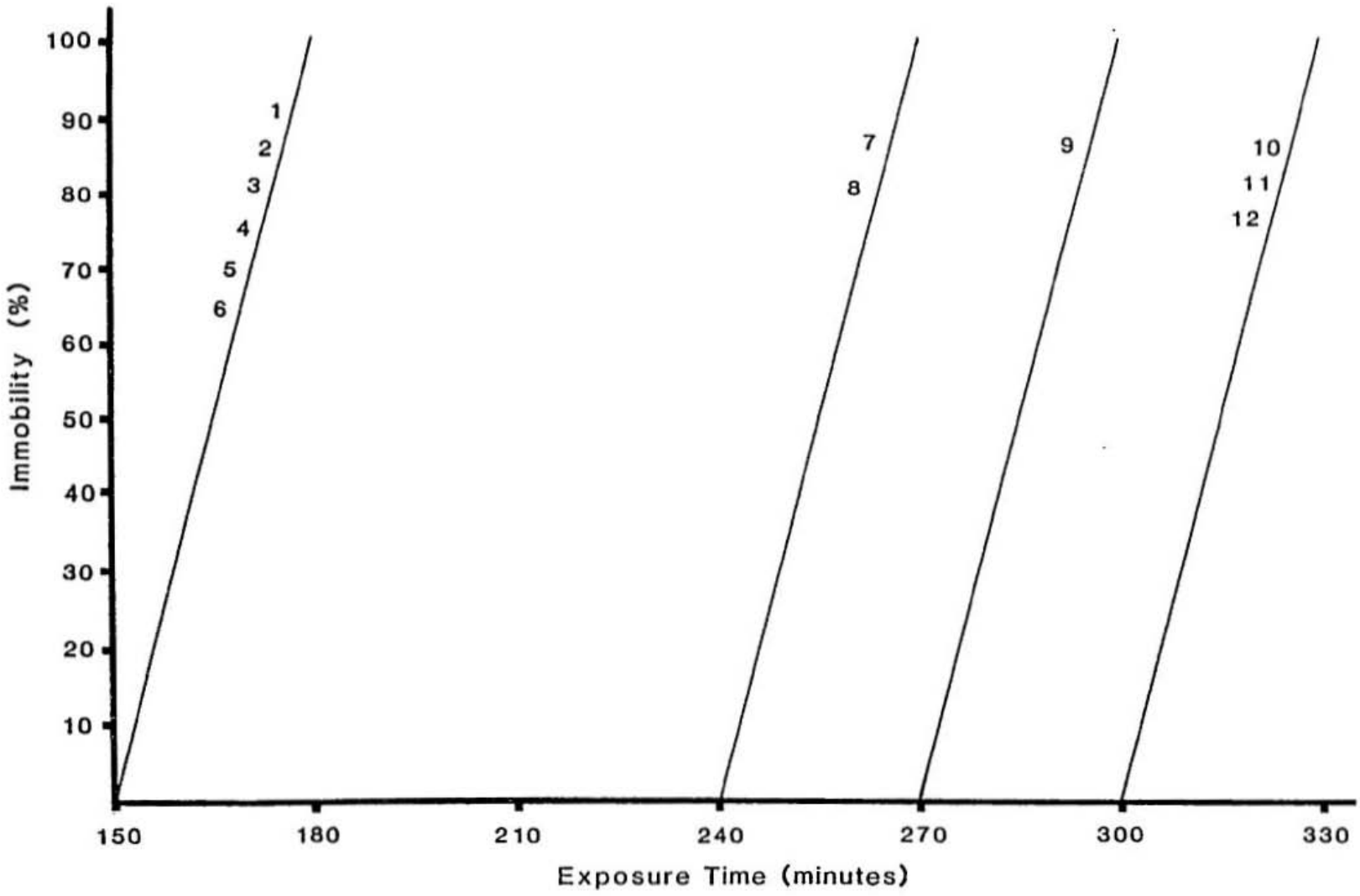
Exposure Time (minutes)

APPENDIX J

The mean percent immobility of L1C immersed in distilled water and additive droplets (conc. 10% additive) and exposed at a saturation deficit of 3.5 millibars for predetermined time intervals. (1 - Distilled water, 2 - Soybean, 3 - Savol, 4 - Cottonseed, 5 - Sunspray, 6 - Citrus, 7 - Klearol, 8 - Pyronyl, 9 - Exxon, 10 - Base, 11 - Heavy, 12 - 2251. Curves are fit by eye for illustrative purposes.)


Immobility (%)
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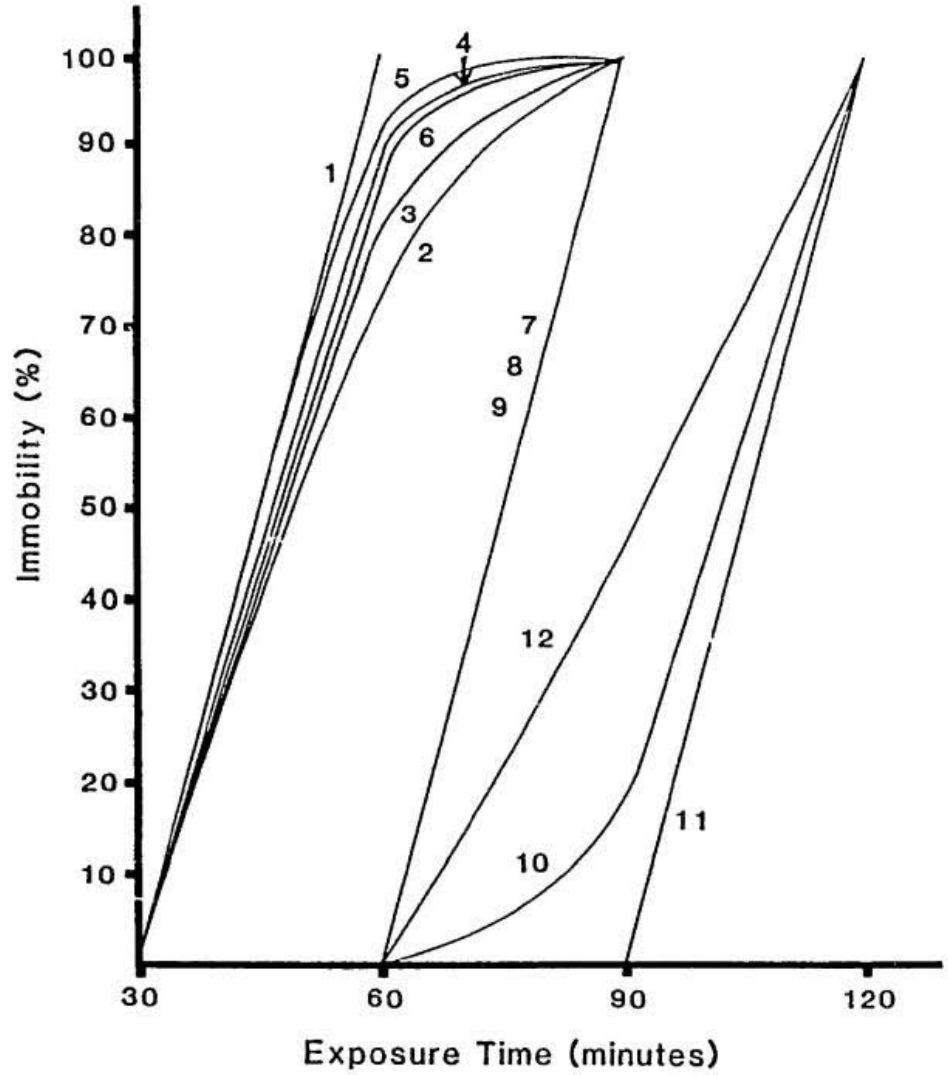
Exposure Time (minutes)

APPENDIX K

The mean percent immobility of All strain immersed in distilled water and additive droplets (conc. 10% additive) and exposed at a saturation deficit of 19.4 millibars for predetermined time intervals. (1 - Distilled water, 2 - Soybean, 3 - Savol, 4 - Cottonseed, 5 - Sunspray, 6 - Citrus, 7 - Klearol, 8 - Pyronyl, 9 - Exxon, 10 - Base, 11 - Heavy, 12 - 2251. Curves are fit by eye for illustrative purposes.)


Immobility (%)
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Exposure Time (minutes)
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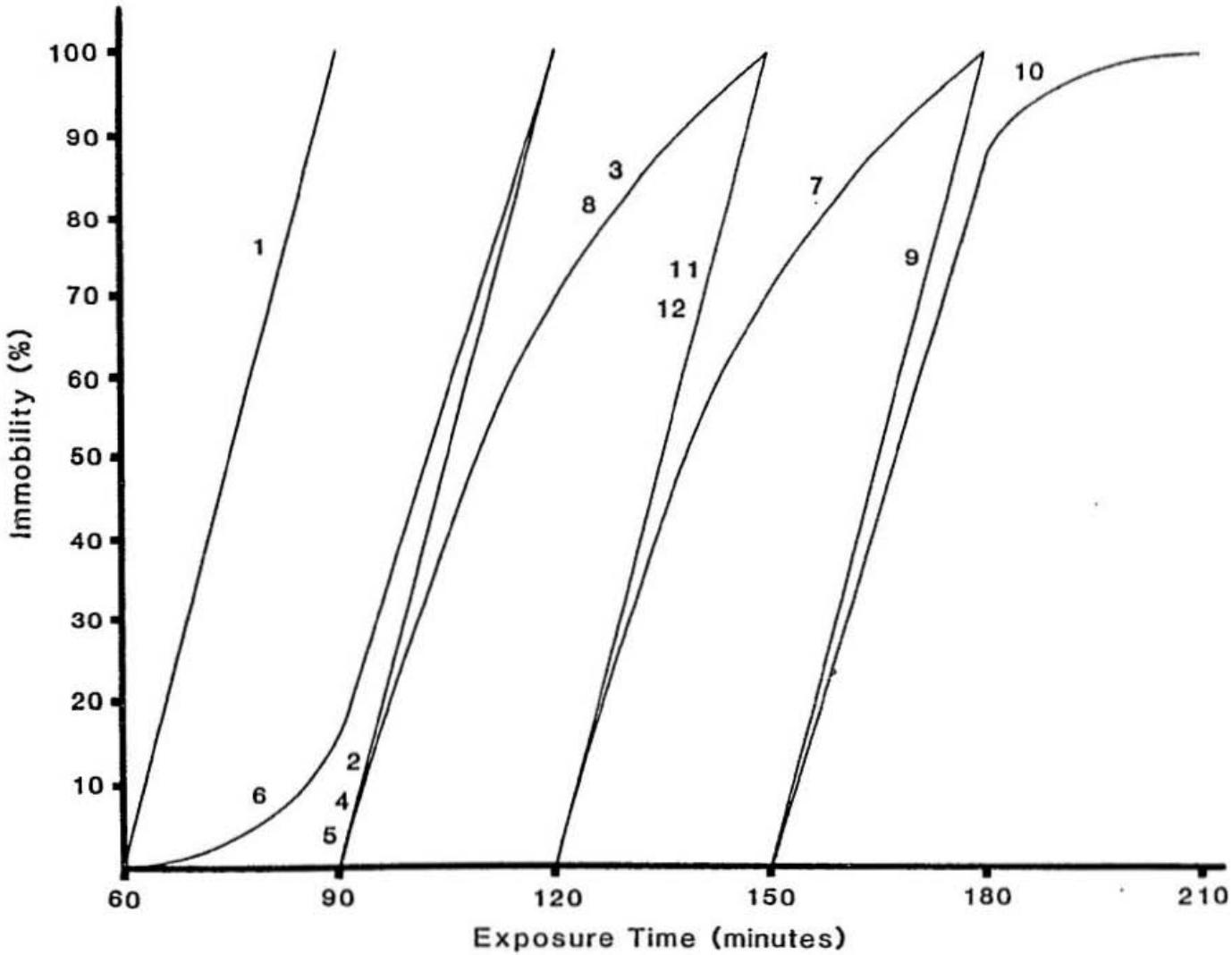
APPENDIX L

The mean percent immobility of All strain immersed in distilled water and additive droplets (conc. 10% additive) and exposed at a saturation deficit of 8.8 millibars for predetermined time intervals. (1 - Distilled water, 2 - Soybean, 3 - Savol, 4 - Cottonseed, 5 - Sunspray, 6 - Citrus, 7 - Klearol, 8 - Pyronyl, 9 - Exxon, 10 - Base, 11 - Heavy, 12 - 2251. Curves are fit by eye for illustrative purposes.)


Immobility (%)
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Exposure Time (minutes)
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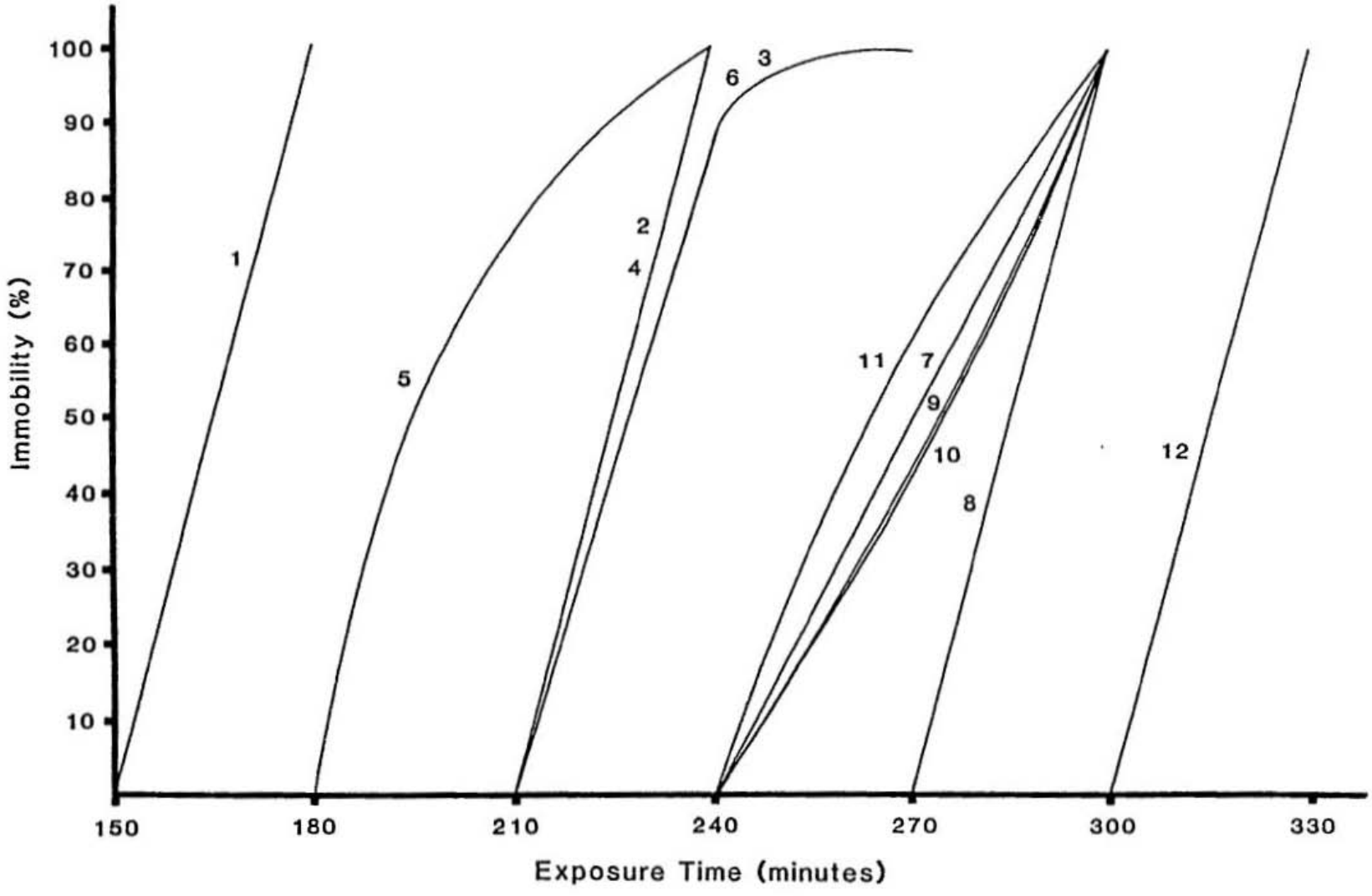
APPENDIX M

The mean percent immobility of All strain immersed in distilled water and additive droplets (conc. 10% additive) and exposed at a saturation deficit of 3.5 millibars for predetermined time intervals. (1 - Distilled water, 2 - Soybean, 3 - Savol, 4 - Cottonseed, 5 - Sunspray, 6 - Citrus, 7 - Klearol, 8 - Pyronyl, 9 - Exxon, 10 - Base, 11 - Heavy, 12 - 2251. Curves are fit by eye for illustrative purposes.)

APPENDIX N

A description of each of the additives used in Part B.


Immobility (%)
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Exposure Time (minutes)

ADDITIVE	DESCRIPTION	REFERENCE
Soybean	Fatty acid composition - linoliec acid 52-60% - palmitic acid 8% - stearic acid 4% - oleic acid 28% - linolenic acid 5% Sterols 0.15 - 0.42% Tocopherols 1000 - 2800 ppm Iodine value 121 - 139% Saponification value 190 - 200 Specific gravity 0.920 - 0.925 at 15.5°C Solidifying point -10 to -16°C	Kirk and Othmer (1983) and Snell and Snell (1962)
Savol	Paraffic oil - 92% Inert ingredients - 8% (Unsulfonated residue - 92%) A medicated soap. Contains salol (phenyl salicylate) with perfumes.	Thompson-Hayward Chem. Co. Kansas City, Ka. 66110 Gardener & Cooke (1978)
Cottonseed	Fatty acid composition - myristic 1% - palmitic 7% - stearic 4% - oleic 26% - linoleic 43% - linolenic 1% Solidfying point - to 0°C Specific gravity 0.922 - 0.930 at 15.5°C Saponification value 191 - 198 Iodine value 104 - 115% Used in soap and cosmetic cream, as a leather dressing and a lubricant.	Kirk and Othmer (1983) and Snell and Snell (1962)
Sunspray 7-N	Light paraffinic mineral oil with straight hydro-carbons of 15 - 30 carbon atoms in length. Low viscosity and volatile. Nonsoluble in water.	Gardener and Cooke (1978)

Klearol	White mineral oil No aromatics Non-soluble Similar to baby oil	Witco Chemical Co.
Pyronyl conc. #360	Pyrethrins 3% Piperonyl butoxide technical 6% N-octyl bicycloheptane dicarboximide - 10% Petroleum distillates 81%	Prentiss Drug and Chem. Co., Newark, N.J. 07105
Orchex Exxon 796	High paraffinic content Pour point -24°C Narrow boiling range	Exxon Co. P.O. Box 2180 Houston, Te. 77001
Base oil C	When semi-drying vegetable oils, marine animals oils and liquid waxes are warmed from 70 - 120°C and a current of air blown through them, the oils oxidise to viscid fluids, miscible with mineral oils. They are rich in triglycerides of the hydroxy-acids, and are used as lubricants.	Gardener and Cooke (1978)

* Information on Citrus, Heavy, and 2251 additives is unavailable.

APPENDIX O

The mean percent immobility of *S. bibionis* immersed in distilled water and additive droplets (conc. 10% additive) and exposed at a saturation deficit of 19.4 millibars for predetermined time intervals.*

Additive	Exposure Time (minutes) 30	60	90
Distilled Water	0(0)	100(0)	
Soybean	34(7)	100(0)	
Savol	28(4)	100(0)	
Cottonseed	26(4)	100(0)	
Sunspray	0(0)	100(0)	
Citrus	0(0)	100(0)	
Klearol	0(0)	69(5)	100(0)
Pyronyl	0(0)	83(0)	100(0)
Exxon	0(0)	100(0)	
Base		0(0)	100(0)
Heavy		0(0)	100(0)
2251		0(0)	100(0)

* Standard error is shown in parentheses after each mean.

APPENDIX P

The mean percent immobility of *S. bibionis* immersed in distilled water and additive droplets (conc. 10% additive) and exposed at a saturation deficit of 8.8 millibars for predetermined time intervals.*

Additive	Exposure Time (minutes) 60	90	120	150	180
Distilled Water	0(0)	100(0)			
Soybean		0(0)	100(0)		
Savol		0(0)	100(0)		
Cottonseed		0(0)	100(0)		
Sunspray		0(0)	100(0)		
Citrus		0(0)	100(0)		
Klearol			0(0)	100(0)	
Pyronyl			0(0)	100(0)	
Exxon			0(0)	100(0)	
Base			0(0)	64(1)	100(0)
Heavy			0(0)	100(0)	
2251			0(0)	67(8)	100(0)

* Standard error is shown in parentheses after each mean.

APPENDIX Q

The mean percent immobility of *S. bibionis* immersed in distilled water and additive droplets (conc. 10% additive) and exposed at a saturation deficit of 3.5 millibars for predetermined time intervals.*

	Exposure Time (minutes)						
Additive	120	150	180	210	240	270	300
Distilled Water		0(0)	100(0)				
Soybean	0(0)	52(2)	100(0)				
Savol	0(0)	52(3)	100(0)				
Cottonseed	0(0)	59(5)	100(0)				
Sunspray	0(0)	51(0)	100(0)				
Citrus	0(0)	72(3)	100(0)				
Klearol					0(0)	100(0)	
Pyronyl				0(0)	37(4)	100(0)	
Exxon					0(0)	100(0)	
Base				0(0)	77(8)	100(0)	
Heavy					0(0)	16(2)	100(0)
2251				0(0)	39(0)	100(0)	

* Standard error is shown in parentheses after each mean.

APPENDIX R

The mean percent immobility of L1C immersed in distilled water and additive droplets (conc. 10% additive) and exposed at a saturation deficit of 19.4 millibars for predetermined time intervals.*

Additive	Exposure Time (minutes) 30	60	90	120
Distilled Water	0(0)	100(0)		
Soybean	0(0)	100(0)		
Savol	0(0)	100(0)		
Cottonseed	0(0)	100(0)		
Sunspray	0(0)	100(0)		
Citrus	0(0)	100(0)		
Klearol		0(0)	100(0)	
Pyronyl		0(0)	100(0)	
Exxon		0(0)	100(0)	
Base		0(0)	100(0)	
Heavy		0(0)	53(5)	100(0)
2251		0(0)	35(7)	100(0)

* Standard error is shown in parentheses after each mean.

APPENDIX S

The mean percent immobility of L1C immersed in distilled water and additive droplets (conc. 10% additive) and exposed at a saturation deficit of 8.8 millibars for predetermined time intervals.*

Additive	Exposure Time (minutes) 60	90	120	150
Distilled Water	0(0)	100(0)		
Soybean		0(0)	100(0)	
Savol		0(0)	100(0)	
Cottonseed		0(0)	100(0)	
Sunspray		0(0)	100(0)	
Citrus		0(0)	100(0)	
Klearol			0(0)	100(0)
Pyronyl			0(0)	100(0)
Exxon			0(0)	100(0)
Base			0(0)	100(0)
Heavy			0(0)	100(0)
2251			0(0)	100(0)

* Standard error is shown in parentheses after each mean.

APPENDIX T

The mean percent immobility of L1C immersed in distilled water and additive droplets (conc. 10% additive) and exposed at a saturation deficit of 3.5 millibars for predetermined time intervals.*

Additive	Exposure Time (minutes) 150	180	210	240	270	300	330
Distilled Water	0(0)	100(0)					
Soybean	0(0)	100(0)					
Savol	0(0)	100(0)					
Cottonseed	0(0)	100(0)					
Sunspray	0(0)	100(0)					
Citrus	0(0)	100(0)					
Klearol				0(0)	100(0)		
Pyronyl				0(0)	100(0)		
Exxon					0(0)	100(0)	
Base						0(0)	100(0)
Heavy						0(0)	100(0)
2251						0(0)	100(0)

* Standard error is shown in parentheses after each mean.

APPENDIX U

The mean percent immobility of All strain immersed in distilled water and additive droplets (conc. 10% additive) and exposed at a saturation deficit of 19.4 millibars for predetermined time intervals.*

Additive	Exposure Time (minutes) 30	60	90	120
Distilled Water	0(0)	100(0)		
Soybean	0(0)	74(4)	100(0)	
Savol	0(0)	81(5)	100(0)	
Cottonseed	0(0)	89(3)	100(0)	
Sunspray	0(0)	92(2)	100(0)	
Citrus	0(0)	87(3)	100(0)	
Klearol		0(0)	100(0)	
Pyronyl		0(0)	100(0)	
Exxon		0(0)	100(0)	
Base		0(0)	18(5)	100(0)
Heavy			0(0)	100(0)
2251		0(0)	47(1)	100(0)

* Standard error is shown in parentheses after each mean.

APPENDIX V

The mean percent immobility of All strain immersed in distilled water and additive droplets (conc. 10% additive) and exposed at a saturation deficit of 8.8 millibars for predetermined time intervals.*

Additive	Exposure Time (minutes) 60	90	120	150	180	210
Distilled Water	0(0)	100(0)				
Soybean		0(0)	100(0)			
Savol		0(0)	69(2)	100(0)		
Cottonseed		0(0)	100(0)			
Sunspray		0(0)	100(0)			
Citrus	0(0)	16(2)	100(0)			
Klearol			0(0)	71(6)	100(0)	
Pyronyl		0(0)	68(6)	100(0)		
Exxon				0(0)	100(0)	
Base				0(0)	87(3)	100(0)
Heavy			0(0)	100(0)		
2251			0(0)	100(0)		

* Standard error is shown in parentheses after each mean.

APPENDIX W

The mean percent immobility of All strain immersed in distilled water and additive droplets (conc. 10% additive) and exposed at a saturation deficit of 3.5 millibars for predetermined time intervals.*

	Exposure Time (minutes)						
Additive	150	180	210	240	270	300	330
Distilled Water	0(0)	100(0)					
Soybean			0(0)	100(0)			
Savol			0(0)	88(3)	100(0)		
Cottonseed			0(0)	100(0)			
Sunspray		0(0)	74(3)	100(0)			
Citrus			0(0)	88(3)	100(0)		
Klearol				0(0)	49(0)	100(0)	
Pyronyl					0(0)	100(0)	
Exxon				0(0)	43(3)	100(0)	
Base				0(0)	42(3)	100(0)	
Heavy				0(0)	60(6)	100(0)	
2251						0(0)	100(0)

* Standard error is shown in parentheses after each mean.

APPENDIX X

The mean elapsed time (seconds) for 9mls of each type of additive and distilled water to dispense from a 10ml pipette at a temperature of 23°C.

Additive Type	Mean Dispensing Time (seconds)	Standard Error
Soybean	21.3	0.0
Savol	16.7	0.3
Cottonseed	11.7	0.3
Sunspray	8.0	0.3
Citrus	8.0	0.0
Exxon	7.0	0.0
Klearol	6.0	0.0
Pyronyl	6.0	0.0
Base	3.0	0.0
Heavy	3.0	0.0
2251	3.0	0.0
Distilled Water	3.0	0.0

APPENDIX Y_1

The confidence limits (in minutes) for the TI_{50} values in Table 2 for *S. bibionis*, L1C and All strain immersed in distilled water and additive droplets (conc. 10% additive) and exposed at a saturation deficit of 19.4 millibars for predetermined time intervals.

	19.4 millibars		
	S. bibionis	L1C	All strain
ADDITIVE	TI50 (95% C.L.)	TI50 (95% C.L.)	TI50 (95% C.L.)
DISTILLED WATER	(34.6–52.1)	(35.7–50.4)	(33.8–53.2)
SOYBEAN	(30.8–31.8)	(35.7–50.4)	(56.3–58.3)
SAVOL	(31.2–32.5)	(35.7–50.4)	(55.5–57.6)
COTTONSEED	(31.1–32.2)	(35.7–50.4)	(53.7–56.1)
SUNSPRAY	(34.6–52.1)	(35.7–50.4)	(52.8–55.4)
CITRUS	(34.6–52.1)	(35.7–50.4)	(54.4–56.7)
KLEAROL	(56.6–58.4)	(69.8–77.3)	(69.9–77.3)
PYRONYL	(54.5–56.5)	(69.8–77.3)	(69.9–77.3)
EXXON	(34.6–52.1)	(69.8–77.3)	(69.9–77.3)
BASE	(71.0–76.1)	(69.8–77.3)	(94.7–98.3)
HEAVY	(71.0–76.1)	(88.5–90.7)	(101.6–106.3)
2251	(71.0–76.1)	(90.4–92.7)	(88.9–91.8)

APPENDIX Y_2

The confidence limits (in minutes) for the TI_{50} values in Table 2 for *S. bibionis*, L1C and All strain immersed in distilled water and additive droplets (conc. 10% additive) and exposed at a saturation deficit of 8.8 millibars for predetermined time intervals.

ADDITIVE	8.8 millibars		
	S. bibionis	L1C	All strain
	TI50 (95% C.L.)	TI50 (95% C.L.)	TI50 (95% C.L.)
DISTILLED WATER	(66.7–81.0)	(76.6–79.9)	(66.4–81.3)
SOYBEAN	(94.7–114.0)	(95.6–113.0)	(96.3–112.1)
SAVOL	(94.7–114.0)	(95.6–113.0)	(116.7–118.5)
COTTONSEED	(94.7–114.0)	(95.6–113.0)	(96.3–112.1)
SUNSPRAY	(94.7–114.0)	(95.6–113.0)	(96.3–112.1)
CITRUS	(108.0–112.3)	(95.6–113.0)	(92.8–94.5)
KLEAROL	(130.6–137.8)	(128.3–140.3)	(145.3–147.6)
PYRCNYL	(135.1–150.4)	(128.3–140.3)	(117.2–118.9)
EXXON	(130.6–137.8)	(128.3–140.3)	(162.1–166.6)
BASE	(146.9–149.0)	(128.3–140.3)	(170.7–174.0)
HEAVY	(130.6–137.8)	(128.3–140.3)	(131.0–137.4)
2251	(145.0–147.3)	(128.3–140.3)	(131.0–137.4)

APPENDIX Y_3

The confidence limits (in minutes) for the TI_{50} values in Table 2 for *S. bibionis*, L1C and All strain immersed in distilled water and additive droplets (conc. 10% additive) and exposed at a saturation deficit of 3.5 millibars for predetermined time intervals.

ADDITIVE	3.5 millibars		
	S. bibionis	L1C	All strain
	TI50 (95% C.L.)	TI50 (95% C.L.)	TI50 (95% C.L.)
DISTILLED WATER	(158.9–169.9)	(157.6–171.3)	(156.0–173.0)
SOYBEAN	(148.9–150.4)	(157.6–171.3)	(219.4–229.7)
SAVOL	(149.1–150.6)	(157.6–171.3)	(233.2–235.5)
COTTONSEED	(148.3–150.1)	(157.6–171.3)	(219.4–229.2)
SUNSPRAY	(149.2–150.7)	(157.6–171.3)	(206.3–208.0)
CITRUS	(147.0–171.3)	(157.6–171.3)	(233.1–235.5)
KLEAROL	(252.4–256.7)	(247.2–262.2)	(269.2–271.1)
PYRONYL	(241.1–243.5)	(247.2–262.2)	(281.9–287.3)
EXXON	(252.4–256.7)	(279.9–289.4)	(269.8–271.8)
BASE	(231.8–234.7)	(311.2–318.2)	(269.6–271.6)
HEAVY	(275.9–279.2)	(311.2–318.2)	(312.3–317.0)
2251	(241.2–243.6)	(311.2–318.2)	(312.3–317.0)