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Integrated Pest Management for Cotton with a focus on Whitefly and Aphids

Integrated Pest Management for Cotton

Final Report for Project CFC/ICAC/03

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ABBREVIATIONS AND ACRONYMS

ARO	-	Agricultural Research Organization of Israel
CCGA	-	Commercial Cotton Growers’ Association of Zimbabwe
CFC	-	Common Fund for Commodities
EARO	-	Ethiopian Agricultural Research Organization
ICAC	-	International Cotton Advisory Committee
IPM	-	Integrated Pest Management
LDCs	-	Least Developed Countries
PEA	-	Project Executing Agency
PCC	-	Project Coordinating Committee
PPRI	-	Plant Protection Research Institute (Egypt)
PY	-	Project Year

FOREWORD

Cotton culture is one of the oldest and most wide spread agricultural practices in the world. Nowadays, cotton serves as a cash crop for both small growers in less developed countries, and for large-scale growers in well developed ones. Over the last century, cotton culture has become one of the most intensively treated crops, spreading to extensive regions of the globe and offering a means of livelihood for millions. Hand-in-hand, numerous negative ecological consequences have stemmed from the increase in insecticide treatments. The absolute increase in insecticide use has been most noticeable in the more developed countries, with the damage being mainly restricted to the environment. In less developed countries, although the environmental damage has been less severe, the direct effect on human health, resulting from direct exposure to poisonous materials, is more pronounced.

The present project was directed towards reducing the use of poisonous materials that are harmful to mankind. This was achieved through: a) the development of new materials that are non-toxic to humans; b) the development of better means of application to bring the materials into direct contact with the pests and reduce insecticide drift; c) examination of the role of natural enemies in the system and their utilization it in lieu of toxicants; and d) the determination of treatment thresholds to facilitate the most efficient use of minimal but effective insecticide treatments.

In addition, the projects served to coalesce cooperation between growers, administrators and research personnel from several countries and continents typified by different growth conditions and approaches. We are hopeful that the bridge thus formed will act as an avenue for continued cooperation.

The project involved extensive expenditure of funds and efforts from numerous persons and agencies, each contributing to its smooth running and success. We wish to thank all those who contributed to the planning and execution of the project:

The Common Fund for Commodities (CFC), that financed it and showed understanding and cooperation in all of its needs.

The project manager Mr. Sietse van der Werff, who spared no time or effort in helping in all matters, large or small in order to facilitate the smooth running and profitable execution of the work.

The International Cotton Advisory Committee (ICAC), that initiated the program for cotton and acted as an intermediate between the participating countries and the CFC and as a professional umbrella organization for our project.

Special thanks are due to Dr. Rafiq Chaudhry, Head of Technical Information Section, who presented the project to the CFC Standing Committee, for his great help in coordinating among all of the agencies and bodies participating in the project. Thanks to Dr. L. Shaw the former head of ICAC who had a keen interest in the project.

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The project ended with the completion of the stated research objective. In order to implement many of its findings, it will still be necessary to invest time and effort in the semi-industrial and industrial development of many of the commercial ideas advanced. All of the participant’s hope that their findings will, indeed, improves the lot of cotton growers throughout the world, especially in the poorer cotton growing countries.

EXECUTIVE SUMMARY

CFC/ICAC/03 project is an international joint effort involving Egypt, Ethiopia, Israel and Zimbabwe, aimed at developing integrated methods for control of whitefly and cotton aphids, both of which cause stickiness to cotton. The broad objectives include the production of high quality non-sticky cotton; increased profitability for both raw cotton producers and processors; and reduction in damage to the environment.

The project developed new target-oriented, environmentally compatible pesticide formulations and their application methods; promoted biological pest control; developed guidelines for economic use of these methods; and disseminated the project findings to extension staff of participating and other countries.

The project comprised five main components:

1. Development of novel target-oriented pesticides.

Target-oriented insecticidal formulations, based on vegetable oils, were developed and tested under controlled conditions. This included: characterization of different species of Vegetable Oils (VO) according to their bioactivities *vs. Bemisia* and their phytotoxic tendencies; accumulating knowledge on the formulation-function relationship of VO as control agents; formulation and scaling up of prototype VO formulations for field application under semi-commercial conditions; development of a laboratory setup for adaptation and quality assurance of formulations based on VO of local origin; and production of experimental VO formulations for field research by the cooperating groups.

The kind of tested VOs, all of major commercial importance, showed a similar activity repertoire, which included toxicological and behavioral components. However, different oils varied in potency, speed of action and bio-persistence, in such parameters as: residual activity against adult and immature *Bemisia*, spray toxicity to larval stages, and modification of adult's behavior expressed by settling and oviposition deterrence. Among the eight tested VOs, groundnut, castor and cottonseed oils showed the most prominent activities. Coconut oil was the most phytotoxic and castor oil was the safest to the crop.

Systematic study of the effects of formulation variables on control-related properties of VOs resulted in several promising formulating procedures. Among these, two formulation lines, one excluding (”optimized”) and the other included an auxiliary component (“stabilized”) gave the most promising results for all the studied VO kinds. Both experimental and field versions of “optimized” and “stabilized” formulations retained VO activity towards the target pest and had minimal phytotoxicity. The formulations made only of non-toxic and environmentally friendly ingredients, were proven to be consistent and stable, to exhibit good dilution stability and were easy to dispense and apply. In addition, a simple laboratory setup was developed and suggested

as a model for development and preparation of similar formulations from local VOs in the target countries.

At the beginning of the project, material No. 4 sprayed by a long drop-tube sprayer (2 treatments at 2-week intervals), caused a reduction of 71.5% on the average infestation level of whitefly immatures on the Acala variety and 50% reduction on the Pima variety. However the long drop-tube sprayer had to be withdrawn due to the damage it caused to the cotton plants, and it was replaced by the Tornado sprayer that was developed within the framework of the project.

66 novel materials were tested in 5 stages, along the course of the project. After each stage, the superior materials were sent for repeat tests to Egypt, Zimbabwe and Ethiopia.

Stage 1: Phytotoxicity was tested and 21 materials were disqualified at this stage.

Stage 2: The remaining 45 materials were tested for their effect on whitefly control, under ideal spray conditions with complete coverage of the underside of the leaves. In this series of tests it was determined that material III gave the best results.

Stage 3: materials III and No. 4 were compared using knapsack and tractor mounted sprayers under standard field conditions. In all of the cases, the results of material III were superior to those of No. 4.

Stage 4: Application methods were tested. It was found possible to reduce spray volume from 500 l/ha to 200 l/ha with material No. 4 if the amount of active material remained constant. In addition, both materials, three tractor-mounted sprayers were compared: the long drop-tube sprayer, the air-sleeve sprayer and the Tornado sprayer. The long drop-tube sprayer had the best results, with no difference displayed between the air-sleeve and Tornado sprayers. Various knapsack sprayers, such as the knapsack drop-tube sprayer, the standard knapsack sprayer and the motorized knapsack sprayer also had good results.

Two applications of material III at 2-week intervals with the Tornado sprayer caused a reduction of average infestation by 55.7% compared with 13.3% obtained under similar spray applications with material No. 4. An additional improvement was obtained with material III by switching to 4 applications at weekly intervals between treatments. Of the three different sprayers (the air-sleeve, the Tornado and the knapsack drop-tube), a reduction of 74% in average whitefly population was obtained in comparison with the unsprayed control under similar conditions. The standard treatment, 2 applications of Diafenthiuron, reduced the population by 86%. There was no difference between the Pima and Acala cotton varieties sprayed by the air-sleeve or the Tornado sprayers.

In a series of four trials in Zimbabwe under various coverage conditions, it was found that a set of novel materials had good aphid control if the underside coverage of the leaves was complete. Under standard field conditions using the knapsack sprayer with tail-boom there were no satisfactory results concerning aphids.

Stage 5: Examining the effects of the materials on beneficial insect populations under field condition will discuss later (in this page).

2. Design of criteria for cotton sprayers and design of new spray techniques.

Three manual sprayers and one tractor-mounted were developed and tested by the engineering groups from Egypt, Zimbabwe and Israel. The sprayers were developed in an effort to achieve very high uniformity of spray deposition, as required for whitefly control with the nontoxic pesticides developed in the present project.

Two motorized Knapsack sprayers were modified for application of the nontoxic pesticides in small farms, one by the group from Egypt and one by the group from Zimbabwe. The group from Israel developed an electrically powered sprayer for the same purpose. Field tests of the manual sprayers showed higher uniformity of spray deposition for all of the newly developed sprayers as compared with commercially available machines.

The tractor-mounted sprayer was developed with a high ground clearance in order to provide high uniformity of spray deposition, while still enabling operation in fields where branches cross from one row to the next. The application tests gave better results than any commercial sprayer known to the researchers, but also gave lower uniformity and lower quantities of pesticides on the targets as compared to an earlier model with very small ground clearance.

3. Biological control.

Our main goal here was to understand the dynamics of the pest and its natural enemies in order to facilitate optimal pest management. This was achieved by determining:

1. The pest biology and population dynamics;
2. The natural enemies' (parasitoids and predators) identity, biology, dynamics and impact - how important are they, and when are they important?
3. The effects of insecticidal (commercially used materials and new preparations) treatments on natural enemies - does it cause damage to control efforts and how can such damage be decreased?
4. The impact of neighboring crops – how do they influence the levels *B. tabaci* populations?

1. Whiteflies were found in all countries throughout the warm seasons. In most cases populations were lowest when cotton was young and rose with the season; a sharp rise in

Egypt and Israel occurred in late July or August. Maximal levels in untreated fields were as low as 2.5/leaf [1997 report, p.194] but also reached as high as 68/leaf [1998 report, p.162-5]. In Ethiopia, high populations were observed early in the season due to their development on alternative hosts from which they had moved on to cotton [1997 report, p.221]. In Zimbabwe, both *Trialeurodes vaporariorum* and *Bemisia tabaci* were active, but did not cause damage in most cases.

2. In Egypt and Israel *Encarsia lutea* Masi and *Eretmocerus mundus* (Mercet) were the predominant whitefly parasitoids [1995 report, p. 101]. In Ethiopia and Zimbabwe *Encarsia transvena* and *Eretmocerus mundus* or a very similar species, were found. Rates of parasitization often rose with the season and reached levels up to 90% [1992 report, p.95; 1998 report, p.199]. The 5-year averages for Israel and Egypt were between 65-70%, while for Ethiopia, the one reported year averaged 47% parasitism. No correlation was found between % parasitism and whitefly population. The use of insecticides did not affect % parasitism in1998 but it reduced parasitism significantly in 1997. The high percentages of parasitism indicate that parasitism alone prevents a significant increase in whitefly populations. This is especially significant in the light of quick resistance build-up in whiteflies resulting from successive treatments with insecticides.

   Many predator species were found. Most coincided among the countries and locations but some did not. The more generally occurring ones were *Chrysoperla carnea*, *Orius* spp. *Deraeocoris pallens* (In Israel), *Campilomma* spp., *Coccinella* spp., *Hippodamia variegata*, *Scymnus* spp., spiders of various kinds and predaceous mites, esp. *Amblyseius swirskii* (studied only in Egypt). Since none of these are neither specific to, nor regular predators of whitefly, we established methods to evaluate their value in our context, drawing correlation between predator abundance and that of whitefly populations, and conducting specific behavioral observations. The only predators showing overall correlation with the whiteflies were species of *Orius*. Studies conducted with *A. swirskii* in Egypt also indicate that the abundance of this species may be linked with that of the whiteflies. Direct observation of predators on plants in relation to whiteflies showed that predators frequent whitefly infested plants. Although this relationship does not always give a clear picture of their activity, the Heteroptera, and probably also the mites, do seem to be important controlling factors of whitefly populations.

3. Predators were more sensitive to insecticides than the parasitoids. Acetamiprid appears to be one of the more harmful insecticides to both parasitoids and predators, with the exception of the predacious mite *A. swirskii* in Egypt. Non-conventional insecticides (e.g. jojoba and mineral oils), and the IGRs: diafenthiuron, Pymetrozine and Pyriproxyfen usually did very little damage to enemy populations while killing high proportions of the pest. Similar results were obtained with Flufinoxiron, Kemesol

(summer mineral oil) and National (winter mineral oil). The same trend was noticed when using natural (vegetable) oils, Bemistop and Buprofezin. Of the insecticides usually used in the cotton field, we found Monocrotophos to be more harmful than Endosulfan. These tests should be continued for more materials and under more varied field conditions.

Materials number 4 and III (developed during the course of the project) were tested by treating selected fields that abounded in natural enemies. Material 4 was relatively innocuous to predators. Material III showed detrimental activity to some of the enemies in the lab and in the field. In the latter case, the damage to natural enemies seemed short lived. The fact that the various materials were found less harmful to natural enemies while still able to control the whiteflies can suggests them as important tools for the control of the pest, especially early in the season.

4. We did not find any advantage in growing corn near the cotton. The main predators therein, *Orius* spp. did not seem to migrate into the cotton. Sunflowers were found to harbor few natural enemies and at times, a lot of whiteflies. Thus at best, the proximity of sunflowers will not cause a whitefly outbreak, but we should not rely on this crop to be a refuge for natural enemies.

4. Establishment of economic thresholds.

Seven field trials were conducted in which a total of 30 populations of whitefly were examined. Each population was counted twice weekly during the 60-day period before 80% boll opening (defoliating day). in the field. Finally, each population was characterized according to the average number of larvae (stage 2 or more, including pupae) per maximum leaf for the entire period. There was a follow-up of the effect of each population on the final yields, yield components, lint quality, sugar level and stickiness. From these populations it was found that the damage threshold on yield and quality averaged between 15 to 20 immatures per leaf. (1999 report, p. 105 Table 15).

Gerling *et al* found that beginning of the season shows migration into the field, but the main increase in population after these results from build-up within the field. Reproduction within the field is dependent on 3 factors:

1. Initial population size.
2. Oviposition and survival of the various stages.
3. Number of generations.

Within the framework of the project, the effect of initial treatment timing which determines the initial population, and the effect of various chemical treatments on survival were studied in relation to very large whitefly populations.

It was found that a series of various treatments reduced the average population throughout the entire counting period at different percentage (Table 2).

Advancing the time of the first treatment, even if the population was very small at that stage, (average of 1.8 immatures/leaf) reduced the population level throughout the entire counting period and average reduction was about 45% for the whole season (1999 report, p. 112 Table 18).

From these results the following conclusions may be drawn:

In fields in which whitefly build-up (migration and development within the field) is slow and can be controlled by 1-2 treatments, a threshold level of 15-20 larvae and pupae may be used.

In fields in which rate of whitefly build-up is high one should speak in terms of "control strategy" and not in terms of a fixed threshold. The aim of this strategy is to ensure that the average population will not rise above 15-20 nymphs/maximum leaf as an average for the entire season. The strategy would include a series of treatments (date and chemical) at **varying thresholds** (from low at the beginning to a higher threshold later on) in order to achieve these results at a minimal cost, while adhering to the correct policy for preventing future pest resistance to the various chemicals. In order to improve preparation of these strategies, this particular study should be continued.

5. Knowledge dissemination.

Knowledge dissemination efforts took place throughout the project and its finalization.

a) Two out of the four PCC meetings took place in Egypt and Israel in the summers of 1996 and 1977, including demonstrations of field trials conducted in the two countries. Extension staff and growers from the two countries attended those demonstrations in addition to the research staff from Zimbabwe and Ethiopia. Field demonstrations of the developed sprayers took place in Zimbabwe (March, 1999) during the third PCC meeting.

b) Two workshops for extension staff and growers were conducted: one in 1998 in Ethiopia (1998 report, p. 263-272) and the second in 1999 in Egypt (1999 report, p. 141-144, Abstract booklet).

c) Two courses for participants from Egypt and Ethiopia were conducted in Israel during the summers of 1997 & 1998.

d) The project and its achievements were presented at two International Conferences: The 2nd International Workshop on *Bemisia*, San Juan, Puerto Rico, June 1998 (1998 report, p.273), and the World Cotton Research Conference-2, Athens, Greece, September 1998 (1998 report, p.275).

e) Five annual professional reports and a final project report were published, including technical appendixes describing the preparation methods for the new insecticides as well as descriptions and assembly instructions for the new sprayers.

f) A guideline manual of the methodology developed was prepared.

Summary overview of budgeted and provided inputs (not final at the time this report was published)

Total Project - ISRAEL

	CFC			Institutes in Israel		TOTAL			
Category	Budget	Contingency	Actual	Budget	Actual	Budget	Contingency	Actual	Utilization
I Civil Works									
II Vehicles and Equipment	220,700	11,035	262,086			220,700	11,035	262,086	113%
III Materials and Supplies	145,000	5,500	119,193			145,000	5,500	119,193	79%
IV Operations and Maintenance									
a Field services	251,600	11,905	274,137			251,600	11,905	274,137	104%
b Other operating expenses	624,000	27,050	646,983			624,000	27,050	646,983	99%
V Training and Workshops									
a Training		5,415				0	5,415	0	0%
b Workshops	53,700	9,095	59,543			53,700	9,095	59,543	95%
VI Staff salaries and Allowances									
a Academics	463,768		497,311	2,215,372	1,933,247	2,679,140	0	2,430,558	91%
b Technicians	25,000		39,399	116,000	104,989	141,000	0	144,388	102%
TOTAL	1,783,768	70,000	1,898,652	2,331,372	2,038,236	4,115,140	70,000	3,936,888	94%

Total Project - EGYPT

Category	CFC		PPRI		TOTAL		
	Budget	Actual	Budget	Actual	Budget	Actual	Utilization
I Civil Works							
II Vehicles and Equipment	160,000	158,198			160,000	158,198	99%
III Materials and Supplies	60,000	55,947			60,000	55,947	93%
IV Operations and Maintenance							
a Field services	116,914	111,506			116,914	111,506	95%
b Other operating expenses	179,560	167,415			179,560	167,415	93%
V Training and Workshops							
a Training	14,290	56,616	15,710	15,710	30,000	72,326	241%
b Workshops	174,121	119,589			174,121	119,589	69%
VI Staff salaries and Allowances							
a Academics			279,000	279,000	279,000	279,000	100%
b Technicians	106,000	102,691	86,000	86,000	192,000	188,691	98%
TOTAL	810,885	771,962	380,710	380,710	1,191,595	1,152,672	97%

Total Project - ZIMBABWE

	CFC		CCGA		TOTAL		
Category	Budget	Actual	Budget	Actual	Budget	Actual	Utilization
I Civil Works					0	0	
II Vehicles and Equipment	19,300	37,045			19,300	37,045	192%
III Materials and Supplies	10,500	3,328			10,500	3,328	32%
IV Operations and Maintenance							
a Field services	29,350	50,686			29,350	50,686	173%
b Other operating expenses	20,000	14,566			20,000	14,566	73%
V Training and Workshops							
a Training	25,000	609			25,000	609	
b Workshops							
VI Staff salaries and Allowances							
a Academics	47,250	24,494	18,250	18,250	65,500	42,744	65%
b Technicians	20,000	10,807			20,000	10,807	54%
TOTAL	171,400	141,535	18,250	18,250	189,650	159,785	84%

Total Project - ETHIOPIA

	CFC		EARO		TOTAL		
Category	Budget	Actual	Budget	Actual	Budget	Actual	Utilization
I Civil Works					0	0	
II Vehicles and Equipment	15,200	13,335			15,200	13,335	88%
III Materials and Supplies	13,000	1,257			13,000	1,257	10%
IV Operations and Maintenance							
a Field services	29,050	16,617			29,050	16,617	57%
b Other operating expenses	20,000	5,959			20,000	5,959	30%
V Training and Workshops							
a Training	25,000				25,000	0	0%
b Workshops							
VI Staff salaries and Allowances							
a Academics	45,000	12,676	14,500	14,500	59,500	27,176	46%
b Technicians	20,000	19,098			20,000	19,098	95%
TOTAL	167,250	68,942	14,500	14,500	181,750	83,442	46%

PART A: DEVELOPMENT OF NEW TARGET-ORIENTED PESTICIDES

Chapter 1: Laboratory studies

Overall Goal:

Development of target-oriented insecticidal formulations, based on vegetable oils

Sub Goal 1:

A. The objective:

Characterization of different species of Vegetable Oils (VO) by their bioactivities, (*Bemisia* as a model pest).

B. The questions asked within the goal:

1. What are the bioactivity modes of VO?
2. How do the bioactivities of VO change with their type?
3. How does the phytotoxicity of VO vary with their type?
4. What is the effective VO concentration ranges for each activity mode?

C. Experiments conducted to answer each question:

1. VO bioactivities: Reports 1995, pp.: 26-31, 33-38; 1996, pp.: 24-25, 28-38, 45-47;
2. Bioactivitis of various oils: Rep. 1995, pp.: 26-31, 33-38; 1996, pp.: 24-25, 30, 33-38;
3. Phytotoxicity of various oils: Reports 1995, pp.: 28-31, 38-40, 42-44; 1998, pp.:17-19, 21-24, 37-53.
4. Concentration ranges used: Reports 1995, pp.: 26-28, 29-31, 33-35;

D. Results and Conclusions:

1. VO bioactivities: All tested VO show several modes of activity including residual activity against adult and immature *Bemisia;* spray toxicity to larval stages, and modification of adult's behavior. There is good correlation between the three modes of activity on adults as affected by oil type and oil concentration, by formulation type and by residue age. VO residues act as residual and spray larvacides. In fact VO residues are more toxic to at least one immature stage than to adults. Immature stages show the same sensitivity order for all VO: 2 $\geq$ 1 > stage 3 >> stage 4 >> eggs.

2. Bioactivities of different oil types: The following table ranks VO according their potency by the various activity modes on *Bemisia*.

Table 1: Bioactivity of vegetable oils on *Bemisia* stages.

TARGET	ACTIVITY MODE	OIL RANKING ORDER[1]
ADULTS (residual effects 2-3d post-spray)	Survival	gr, co > cas > can > cor, soy > saf, sun > coc
	Settling deterrence	gr, co > cas > can > cor, soy > saf, sun > coc
	Oviposition deterrence	gr, co > cas > can > cor, soy > saf, sun > coc
ADULTS (residual)	Speed of action	co ≥ soy > cas
	Bio-persistence	cas > gr> can, cor > co > soy > saf, sun
IMMATURES	(survival)	
	Residual	cas > gr > can, co, cor >saf, soy, sun
	Direct spray (larvae)	gr > co > soy > cas, can, corn, saf, sun
	Direct spray (egg)	gr, cas > can, co, cor, saf, soy , sun

1 castor, canola, corn, cottonseed, coconut, groundnut, safflower, soybean, sunflower

3. Phytotoxicity of various oil types: Properly formulated, except from coconut oil, VO are usually safe to plants. Under extreme conditions VO species can be ranked by their phytotoxicity as follows: coc >> gr > canola, corn, co, saf, sun, soy > cas.
4. Effective concentration ranges: Residues of 0.3% VO of all species (sprayed to runoff) are non-active 2d post-spray. On the other hand, after the same aging interval, 3% VO residues result in total or nearly total kill of and prevent oviposition.

   Direct spray 3%-VO kills larval stages I, II and III, while the percentage of survival of pupae ranged between 20 and 60%, according to the oil type. Eggs are the most tolerant stage to direct sprays, and their %-survival ranges are 20-100% and 10-60% for 3%- and 5%-applied concentration, respectively.

E. Lesson learned

1. VO bioactivities:
   a. A similar activity repertoire, which includes toxicological and behavioral components, characterizes VO of major commercial importance, notwithstanding differences in their composition and chemico-physical properties.
   b. Residual activity against adults relied mainly on continuous and persistent settling deterrence, which prevented oviposition, and caused adult death due to dehydration and starvation.
   c. VO bioactivities appear to rely on attributes, which are common to oils of different composition and physical properties. The triglyceridic fraction appears to be the main active ingredient.
   d. The various activities of a VO should be weighed and considered as a factor in selection of application method and timing, according to crop requirements and the conditions prevailing in target countries.
2. Bioactivities of different oil types:
   a. Different oil types constitute alternatives to each other. Nevertheless, the specific activity profile of a VO should be considered during selection of the applied concentration, spraying intervals etc.
   b. Groundnut and cottonseed oils appear to be the most effective oil kind for short-term activity on adults, and for direct activity on larvae.
   c. Castor oil, followed by groundnut oil, produce the longest residual activity against adults and some of the immature stages.
   d. Cottonseed oil residues are fast acting, and result in considerable % mortality of adults after few exposure hours.
3. Phytotoxicity of various oil types: Most VO can form formulations that are safe to the crop. Coconut oil, a waxy lipid under ambient temperature, is an exception and tends to cause leaf-burns. On the other hand, castor oil is significantly safer than the other studied VO.
4. The effective concentration ranges: The effective concentration range can be associated with a mechanism of a “physical barrier” and a lack of biochemical toxicity. The relatively high quantities of VO needed for obtaining high mortality can be regarded as a “payment” for its safety, and encourage manufacturing in the target countries. The local industries should know how to evaluate the control properties and how to formulate the available VO species.

Sub Goal 2:

A. The objective:

1. To design and scale up production of VO formulations for field evaluations
2. To improve knowledge on the relationship Formulation-Function of VO as control agents

B. The questions asked within the goal:

1. What are the main requirements from the VO formulations?
2. What are the applicable approaches to develop suitable VO formulations?
3. How do process parameters affect the properties of VO formulations?
4. How do composition factors affect the properties of VO formulations?
5. Based on the acquired knowledge, what are the best types of VO formulations?

C. Experiments conducted to answer each question

1. The requirements: -refer to particular experiments in each report.
2. The approaches: Reports 1995:pp 26-44; 1996:pp 24-48, 50-65, 71-85; 1997:pp 10-19, 20-43, 52-77, 95-126; 1998:pp 14-25, 29-53, 60-69, 80-120, 290-293.
3. Process effects: Reports 1996:pp 25-32, 39-47; 1997:pp 11, 17-19, 22-30;
4. Composition: Reports 1996:pp 26-29,41-37; 1997:pp11-15, 17-19, 22-43; 1998:pp 14-40, 45-53.
5. Recommended formulations: Reports 1998:pp 60-69, 290-293.

D. Results and Conclusion

1. The requirements: a VO formulation is considered successful if it:
   a. Allows the full activity against target pests to be realized.
   b. Minimizes phytotoxicity.
   c. Consists only of non-toxic and environmentally friendly ingredients.
   d. Is consistent and stable.
   e. Is easy to dispense and to apply; and exhibits good dilution stability.
   f. Is easily adaptable by the industries in the target countries and provides flexibility in manufacturing and optimum economics.
2. The Approaches: The relevant properties of the VO formulations can be governed by process and composition variables. Process parameters include the equipment (mixers, mixing vessels), mixing speed and duration, temperature programming, mode of addition of ingredients, and other operational factors. Determining compositional factors include the types and concentrations of the primary

emulsifiers, coemulsifiers (chemicals that reduce interfacial tension during emulsification and ensure long-lasting surface films on the oil droplets), consistency increasing agents (polymers whose chemical nature makes them particularly suitable for stabilizing oil emulsions; they can act both by being absorbed by the oil droplets and as viscosity-controlling agents), solvents, and chemical stabilizers (chemical that delay spoilage of vegetable oils).

3. Process effects: The stability of VO emulsions can be enhanced by energy investment through mixing efficacy, mixing speed, mixing time and temperature. The required stability for the scaled up experimental field formulations, can be obtained by optimization of process variables through utilization of a rotor-stator homogenizer, appropriate mixing vessel and a procedure that includes phase inversion, dropwise addition of the water phase, temperature programming and sometimes also mechanical separation.

   The procedure, which does not require auxiliary stabilizers, was found to be applicable to eight types of VO (named ”optimized VO emulsions”). The optimized emulsions are long-term stable, safe to the crop, exhibit good dilution stability, and are easy to dispense and to apply. The biological activities of a given VO on a target-pest are not affected significantly by variation of the process variables.
4. Composition factors: The concentration and type of the primary emulsifier strongly affect emulsion stability and phytotoxicity. Although Tween-80 does not guarantee the best results for either stability or safety, when the two parameters are considered, this emulsifier is the best choice among the studied biocompatible surfactants. Field formulations of adequate stability and consistency can be achieved by inclusion of auxiliary agents in the VO emulsions. Among these, addition of acetyl alcohol (“a solid coemulsifier”) results in the most stable emulsions, which are characterized by low phytotoxicity and high resistance to aging effects. Cetyl alcohol is commercially available, is not expensive and its environmental friendliness and its safety to humans are well established. The other compositional variants of the oils emulsion are less stable (e.g. in the presence of sodium carboxy methyl cellulose or ascorbic acid) or more phytotoxic (e.g. emulsions that include nolanol, ascorbic acid with and without stabilizers, polyvinyl alcohols, linoleic acid) or both.
5. Recommended formulation mode: Optimized and Stabilized oil emulsion types give the most promising results under laboratory conditions. Optimized VO emulsions which are prepared in the absence of any auxiliary ingredients give good and consistent performance but their preparation on large scale might require

comparatively expensive equipment. The second emulsion variant, Stabilized VO emulsions, which contain 0.2%-cetyl alcohol show good resistance to aging effects.

E. Lesson learned

1. The requirements:
   a. The phrased requirements provide helpful guidelines for screening and evaluating of different formulation modes for VO.
   b. Based on these requirements, emulsifiable concentrates appear to be the most suitable formulation mode for VO. It is noteworthy that emulsifiable concentrates are the principal type of non-aqueous agricultural formulations, and offer maximum flexibility of manufacture. They are considered easy to develop and to adapt.
2. The Approaches: Both approaches have their pros and cons. Optimization solely by procedure avoids possible complications due to undesired intrinsic properties of the auxiliary components such as phytotoxicity and antagonistic activity. On the other hand, emulsions that contain appropriate concentration of auxiliary components may be less expensive and show long-term consistency and stability. This is especially advantageous for large-scale preparations, as energy demands are steeply increased with the produced quantities.
3. Process effects: Pertinent investment of energy during the emulsification can produce VO formulations of promising properties, which consist only of the active ingredient and biocompatible surfactants. Even distribution of small sized oil droplets appears to be associated with the favorable properties of this emulsification mode.
4. Composition factors: The performance of VO emulsions can be highly susceptible to the presence of auxiliary components. The effects of physical and chemical stabilizers may be contradictory, and each can be accompanied by enhanced phytotoxicity or modified activity on target pest. The VO performance in the presence of auxiliary components is sometimes highly sensitive to the preparation mode.
5. Recommended types of VO formulations: The final selection of the oil kinds and the emulsification mode should be made at the target country according to economical and manufacturing considerations. Among the studied VO emulsions, those based on castor oil are the most stable and safe to the crop, and give the longest duration of residual activity. This oil type may be a good candidate for the “optimized” technique. On the other hand, VO species such as sunflower oil, or

even cottonseed oil, which are inherently less stable may exhibit better performance in “stabilized” emulsions [1998 report, pp. 12-57, graphs 1,2,5,7-12].

Sub Goal 3:

A. The objective:

Recommendations for the laboratory setup for development and quality assurance of VO formulations.

B. The questions asked within the goal:

1. What are the recommend procedures for preparation of VO emulsions?
2. What are the recommend methods for stability assessment of VO emulsions?
3. What are the recommend methods for assessment of VO bioactivities on target pest?
4. What are the recommend methods for assessment of VO phytotoxicity tendency?
5. What are the recommend methods for development of novel VO formulations?

C. Experiments conducted to answer each question

1. Recommended emulsification procedures: reports 1995 pp.41-44, 1996 pp.24-29, 1997 pp.11-17, and 1998 pp.14-19.
2. Stability assessment: reports 1995 p.26, 1998 p.13.
3. Activity on target pest: reports 1995 pp.25-26, 1996 pp.22-23, and 1997 p.10.
4. Phytotoxicity tests: reports 1995 p.26, 1996 pp.22-23, 1997 p.10, 1998 pp.12-13.
5. Development scheme: 1997 report, pp.8-9.

D. Results and Conclusion

1. Recommended emulsification procedures
2. Stability assessment: Appendix 1, item 3.
3. Activity on target pest: Appendix 1, item 4.
4. Phytotoxicity tests: Appendix 1, item 5.
5. Development scheme: The following guidelines are suggested for development of target-oriented, insecticidal emulsions, based on vegetable oils, at the target countries:

Table 2: Guidelines for developing of vegetable oil

STAGE	FORMULATION STUDIES	ASSAYS	REMARKS
1- Simple emulsions	Screening of primary emulsifiers (type & concentration)	VO characterization by Activity profiles Phytotoxicity tests	1) The emulsions contain only VO+emulsifier + water. 2) Requires small amounts of emulsions
2- Experimental formulations	Process variables Composition variables	Stability Sprayability Activity profiles Phytotoxicity tests	Small scaled preparations
3- Scaling up	Process variables -- mainly	Shorten quality tests (and Field tests)	1) Scaling up of the most promising procedures 2) Quality tests preformed on each preparation batch prior to field application.
4- Optimization and long-term stability	Process variables -- mainly	Quality tests Long-term stability studies (further Field tests)	1) Further optimization and stability studies as required by field trials.

E. Lesson learned

1.-5. Recommendations for laboratory setup: VO are heterogeneous mixtures whose composition varies between and within kinds, between manufacturers and even between batches. Moreover, the bioactivities of VO occur probably by contact, hence VO performance is strongly influenced by their formulation and/or application mode.

Therefore, a field predictive laboratory setup for characterization of VO formulations is an essential tool in the development and quality assurance of VO as pest control agents.

The purpose of the suggested simple laboratory setup is to serve as a model for development and preparation of VO formulations at the target countries.

Chapter 2: Field trials

1st. Trial objectives:

To test if the novel environmentally friendly materials that were developed in the laboratory are controlling whitefly and aphid under field conditions and are not detrimental to yield and lint quality.

B. Sub goals - Questions asked in order to meet the field trial objectives:

1. Are the materials phytotoxic as seen through leaf scorching, growth retardation, color change or yield reduction?
2. Do they control various whitefly or aphid stages under optimal spray conditions?
3. Can effective control results be obtained under standard treatments using knapsack or tractor mounted sprayers?
4. What is the optimal application method? Spray volume per hectare, concentration of active material and type of sprayers?
5. Do they affect beneficials?

C. Trials conducted to answer those questions:

Egypt	1996 report, pages: 49-99; 128-130
	1997 report, pages: 91-94
	1998 report, pages: 62-70; 71-79
	1999 report, pages: 22-25
Israel	1995 report, pages 45-57; 58-66
	1996 report, pages 67-89; 90-97; 98-127
	1997 report, pages 51-77; 78-90
	1998 report, pages 58-61; 153-156
	1999 report, pages 10-21
Zimbabwe	1997 report, pages 118-126
	1998 report, pages 80-94; 95-116
	1999 report, pages 26-32; 33-40
Ethiopia	1998 report, pages 117-120

D. **Results** (Re: subgoals):

1. Phytotoxicity

Tests for phytotoxicity were conducted under severe application conditions of up-to run-off with 3% active material concentration at 2-week intervals. The effects of scorching color change and growth retardation were examined at 4 stages of plant development.

A total of 66 different materials were tested. Of these, 21 showed various degrees of phytotoxicity and were discarded. The remaining 45 materials were tested for effectiveness of whitefly and aphid control. The materials that were found to have no phytotoxic effects were sent to Egypt, Zimbabwe and Ethiopia for further testing, and included among others 18 oil formulations based on 8 different vegetable oils and 4 experimental preparation methods. In field trials in the different countries, no phytotoxicity was found for these formulations except for a preliminary formulation based on cotton oil that showed phytotoxicity and reduced the yield during the first trial season in Egypt [report 1996, pp. 49-66].

2. Pest Control.

a. Whitefly:

45 novel materials that were found to have no phytotoxicity were tested.

3 comparisons were conducted for various application methods. Each treatment was applied twice at 2-week intervals. [1997 report, pp. 51-57].

1. Leaf dipping (without removal from plant) in a 3% concentration.
2. Spraying the underside of individual leaves in a 3% concentration.
3. Knapsack spraying of entire plants to run-off.

The dipping results were the best. The less effective results were obtained using the knapsack sprayer to run-off. The materials were rated according to their effectiveness in whitefly control.

The 13 superior materials were re-tested one year later, changing spray methods from 2 bi-weekly treatments to 4 treatments with 10-day intervals between each. Using this method, good results were obtained for all materials, even when applied with a standard knapsack sprayer.

The most effective material found was No. III.

Of the oil formulations developed during the course of the project, the formula based on cotton oil was the most efficient for reducing the whitefly population when there was complete leaf coverage [1997 report, pp.61-65, 1998 report p. 61, Cotton oil formulations are marked A or C], [Egypt 1999 report p25, table 2].

b. Aphid control.

Aphid control was tested in 4 trials in Zimbabwe 1997/98, [1998 report, p. 95]. Cotton grown in Zimbabwe is of the *Hirsutum* types. Eleven of the new materials were tested using 4 different methods of application. Complete and rapid control was attained with all of the materials when the underside of the leaf obtained perfect coverage. The fact that the aphids began to reappear several days later proved that the materials have no lasting effect. Whenever coverage was incomplete, control was less effective. The poorest results were obtained using a knapsack sprayer with a tailboom, which i.e. the application most similar to the commercial treatment.

In a repeat trial conducted in Zimbabwe in 1998/99 using 2 of the same materials and 2 different sprayers (knapsack with tailboom and a new knapsack drop-tube sprayer developed for the project) no control was attained despite the fact that 4 weekly applications were given. [1999 report, p.39 fig.4]

In Egypt 6 materials based on vegetable oils effectively controlled aphid populations [1996 report, table 14, p. 65]. In a repeat trial conducted in Egypt in 1999, good control was achieved by the 6 tested formulations including those based on cotton and castor oils with or without stabilization [1999 report, p.24, table 1].

3. Sprayers efficacy.

Four sprayers were tested in Israel in order to evaluate the results achieved using the novel insecticides under standard field conditions: The conventional tractor mounted air-sleeve sprayer commonly used for pest control in cotton, and 3 sprayers that were developed during the course of the project (the long drop-tube sprayer and the Tornado sprayer, both mounted on tractors) and the new knapsack drop-tube sprayer. We tested under field conditions materials that proved to be highly effective in previous trials. These included No. 4 that was used in the first 3 years of the project; it was compared to material III during the 4th and 5th year.

In all of the above trials, the materials were compared with an untreated control and the standard treatment used today in Israel. The new materials were applied twice. The first application was made according to the threshold level of 6 immatures per maximum leaf and the second application was given 2 weeks later.

Satisfactory results were obtained in the 1995 and 1996 trials conducted using material no. 4 with the long drop-tube sprayer in the Acala variety in the southern coastal region in Israel. However, trials with this material in Pima cotton in 1996-1998 were unsatisfactory. Better results, though still not good enough, were obtained using material III.

In 1999, material III was again used when applied in 4 sprays at weekly intervals using 3 different sprayers: the air-sleeve sprayer, the Tornado sprayer and the knapsack drop-tube sprayer. The trial was conducted in the northern part of the coastal region in the Acala and Pima varieties. Results were poorer than the standard treatment (2 Diafenthiuron applications).

Four materials were tried in Zimbabwe (cotton oil, Castor oil, formulae 4 and III), using the knapsack sprayer with the tailboom, in 4 weekly applications in comparison with the control and Buprofezin spray (Glendale 98/99). The results using all of the 4 novel materials were similar to the Buprofezin treatment with significant reduction of whitefly populations in comparison with the control [1999 report, p.32, table 6].

Similar results were obtained the same year in the trial with the Tornado sprayer in Kadoma, Zimbabwe.

In Egypt in the 1999 trials, good whitefly and aphid control was obtained using a knapsack sprayer with 6 novel materials including those based on cotton and castor oils with or without stabilization [1999 report, pp.22-25].

4. Tests for combined optimal application, spray volume, amount of active material and sprayer type.

   This aspect was tested in Israel in four different trials:

   In 1996, the trial was conducted using the long drop-tube sprayer in comparison with the air-sleeve sprayer, using material no. 4. Various combinations of 500 liter per hectare in comparison with 200 liter per hectare with amounts of active ingredient (a.i.) of 6 and 15 liter per hectare were tested. Irrespectively spray volumes of 15 liter, gave significantly better results than 6 liter per hectare, and the long drop-tube sprayer gave better results than the air-sleeve sprayer [1996 report, p. 183, table 2].

   In 1997 the long drop-tube and Tornado sprayers were compared using material no. 4. The long drop-tube sprayer gave significantly better results than the Tornado sprayer [1997 report, p. 86, table 5].

   In 1998 the long drop-tube sprayer was compared with the Tornado sprayer using 2 different materials. The former performed better with both materials [1998 report, p. 222, table 1].

   In 1999 three sprayers were compared: The air-sleeve sprayer, the Tornado sprayer and the new knapsack drop-tube sprayer that was developed for this project. Each sprayer was used for 4 weekly applications of material III in a trial conducted in the northern coastal region with both, Acala and Pima cotton. There were no differences among the 3 sprayers. We also compared, in Acala cotton, two bi-weekly and four weekly spray regime. The 4 treatments were superior to 2 treatments [1999 report, p.17, table 8].

   Summary: Using these materials, the quality of coverage of the under-side of the leaf is of much importance. The long drop-tube sprayer had better coverage and gave superior results than the other sprayers, but this sprayer caused much damage to the plant foliage and so its use was discontinued.

The air-sleeve and Tornado sprayers had poorer results, but this may be improved by applications of 4 weekly treatments. The 15 l/ha amount of active ingredient (material no. 4) was superior to 6 l/ha, but the spray volume could have been reduced from 500 l/ha to 200/ha provided the amount of active ingredient was kept constant.

Conclusions

There were great differences between years in the quality of the results, with good results in the first two years trials using the Acala variety in the southern region in comparison with the less satisfactory results later on obtained from trials in the Pima variety in the northern coastal region. In addition to the difference between varieties, the much greater infestation levels of whitefly than the southern region characterize the northern coastal region.

Table 1 summarizes population reduction of whitefly nymphs using the novel materials, in comparison with the control and with Diafenthiuron treatment (as a standard).

The following calculations used:

1. In each trial, the average population for each treatment during the entire counting period was calculated.
2. For each treatment, the average population was calculated as the percent of the unsprayed control population in the same trial.
3. The average percentages obtained in this calculation were deducted from 100% to show the reduction percent in the said treatment with relation to the appropriate unsprayed control.

The reduction in the infestation level is a function of the stages that are controlled by the material and of the percent of control of the same stages that the material affects. With novel materials that were developed within the boundaries of this project, the results were affected by the coverage quality on the under-side of the leaf. For this reason, the best results obtained under field conditions were with the long drop-tube sprayer. Unfortunately, the long drop-tube sprayer caused much damage to the cotton plants, particularly of the Pima variety (plants with tangled foliage) and was therefore rejected. The differences in reduction of populations with this sprayer in the Acala variety when compared with Pima may be found in the comments below table 1.

The Tornado sprayer couldn't improve results when compared with the standard air-sleeve sprayer. Improvements gained using material III which is more efficient than Formula no. 4, and performing 4 weekly applications rather than 2 that were made in the first 4 years of the research.

The final reduction of the average population by 74% obtained from the three different sprayers with material III in the northern area, is even superior to those gained at the southern using of the long drop-tube sprayer with material no. 4 (71.5%). However the price paid is the increased number of spray applications from two to four.

In the Glendale, Zimbabwe trials during the last year of the project, four treatments were applied with one-week interval between applications. Good whitefly control was achieved using cotton oil and castor oil using the knapsack tailboom sprayer.

In Egypt good whitefly and aphid control was achieved with four applications of material III, IV, stabilized cotton oil and optimized cotton oil when using the knapsack sprayer [1999 report, pp. 22-25].

Chemical control of whiteflies based on a strategy that includes choice of application dates and the materials. These strategies must be adapted to local conditions, such as infestation levels, regional policy for prevention of resistance development, cost, etc. Table 1 presents a selection of treatments (type of sprayer, materials, amount of active material and number of applications) and the percent of population reduction throughout time, that were attained from these treatments in trials conducted during this project. This table may be used as guideline in different regions for choosing specific strategies suitable to local conditions, taking into consideration the control efficiency, cost, resistance, and toxicity to humans. The strategies will be different for different conditions. In regions with low infestation, maybe 2 treatments with material III that would reduce the population by 56% when compared with control would be sufficient. In regions with severe infestation, it is possible that a reduction of 74% that is achieved by 4 treatments is insufficient. In the standard 2 Diafenthiuron treatments the reduction was 86%, that is, the remaining population was 14% of the untreated control. 74% control means that 26% of the pest population in the control remained in the field. In these cases, a combination of early treatment with Diafenthiuron for reducing the population and further treatment with material III would be a possible solution.

It should be stressed that the materials developed during the course of the project that are based on vegetable oils may perform a function similar to formula III as they act by contact. Because of the manner in which they operate, one could assume smaller probability of resistance buildup in compare to metabolic insecticides. The oil materials have an advantage over material No. 4 or III, as it is easy to prepare them from local components in the prospective countries according to the formulae developed within the framework of this project.

Several oils were found to be efficient against whitefly and aphids, including cotton, castor, canola and soybean oils. These formulations were prepared using a simple method, retained their stability and efficiency for several months and had no phytotoxic effects on the cotton plant. Of these formulations, those based on cotton oil excelled.

The importance of good leaf coverage should be stressed when using these materials. The continuation of their development should include this factor.

Table 1: Reduction of immature whitefly populations (calculated as seasonal averages) as percentage of the relevant control

				Tzora	Gvir	Hahotrim	MM 97	MM 98	MZ A 99	MZ P 99	Average
Unsprayed control Seasonal average larvae/maximal leaf				22.1	22.4	42.3	99.9	179.6	37.3	123.6	
Sprayer	Material	No. sprays	a.i./l/ha	% reduction							
Air-sleeve	Diafenthiuron	2	1				82.0	86.0	91.0	86.0	86.0
	4	2	15		57.6						57.6
	4	2	6		35.7						35.7
	III	4	15						74.5	73.1	73.8
Long drop-tube	4	1	15	28.5							28.5
	4	2	15	71.0	71.9	53.9	47.4	49.4			58.7*
	4	2	6		60.3						60.3
	III	2	15					69.4			69.4
Tornado	4	2	15				6.1	20.4			13.3
	III	2	15					47.0	64.3		55.7
	III	4	15						78.3	69.4	73.9
Knapsack	III	4	15						78.8		78.8

* Average of 71.5% for Acala in the southern region and 50.2% for Pima in the northern region.

PART 2: DESIGN OF CRITERIA FOR A COTTON SPRAYER AND DESIGN OF NEW SPRAY TECHNIQUES

Chapter 3: Development of tractor mounted and knapsack prototype sprayers

A. Goal

To develop a pedestrian carried sprayer and a tractor mounted sprayer that would meet the requirements and optimize the application of pesticides developed in the project.

B. Questions asked within the goal:

These pesticides, which are non-toxic to mammals, found to be effective on whitefly only when the spray deposition was of relatively very high uniformity, high volumes of liquid and active material. Since it is inefficient to carry and spray large quantities of water and very costly to use large quantities of active material, the optimal quantities were investigated. A typical volume of 400 liter per hectare and 16 liter per hectare of active material were determined. Within these requirements the pedestrian-carried sprayer and the tractor-mounted sprayer were developed.

C. Experiments conducted to answer each question

1. General

A tractor mounted and three backpack cotton sprayers were developed and tested by our groups, in an effort to achieve the high uniformity of spray deposition required for control with the new pesticides developed in the present project. The first tractor-mounted sprayer had long drop tubes and provided very high uniformity in addition to high droplet density. However, due to its low ground clearance, it presented difficulties where branches were crossing from one row to the next. The second tractor-mounted sprayer was developed to provide higher ground clearance, but the application tests showed slightly lower deposition uniformity. Two motorized backpack sprayers were modified for application of the nontoxic pesticides in small farms, one by the group from Egypt and one by the group from Zimbabwe. The group from Israel developed an electrically powered sprayer for the same purposes [1998 report, p. 122; 1999 report, p. 43].

2. Research methodology

Preliminary spraying experiments with certain nontoxic pesticides conducted in Israel from 1989 until 1994 showed that coverage provided by the commercially available sprayers was insufficient for satisfactory control even under moderate infestations. Based on these experiments, the first tractor mounted sprayer (Mark I) was constructed in 1994, with very long drop tubes. The ground clearance of this sprayer was typically 10 to 15 cm. [1995 report, p. 67 and Figure 1].

Figure 1: The Mark I prototype sprayer with long drop-tubes, inside a cotton field.

The Mark I sprayer provided very good coverage uniformity as shown below and, also, good results in controlling moderate infestation of whitefly in cotton fields. However, when experiments were conducted in Pima cotton with branches crossing from one row to the next, the ground clearance of 12 cm was insufficient and the drop-tubes caused damage to branches or in other cases the drop tube was lifted and the spray was missing some targets. Based on these findings the engineering groups in Egypt, Zimbabwe and Israel developed three backpack sprayers, one in each country, [1999 report,p.49] and a tractor-mounted sprayer with high ground clearance that was developed in Israel [1996 report, p. 133; 1997 report, p. 129].

D. Results

1. Development of an under the canopy sprayer in Egypt

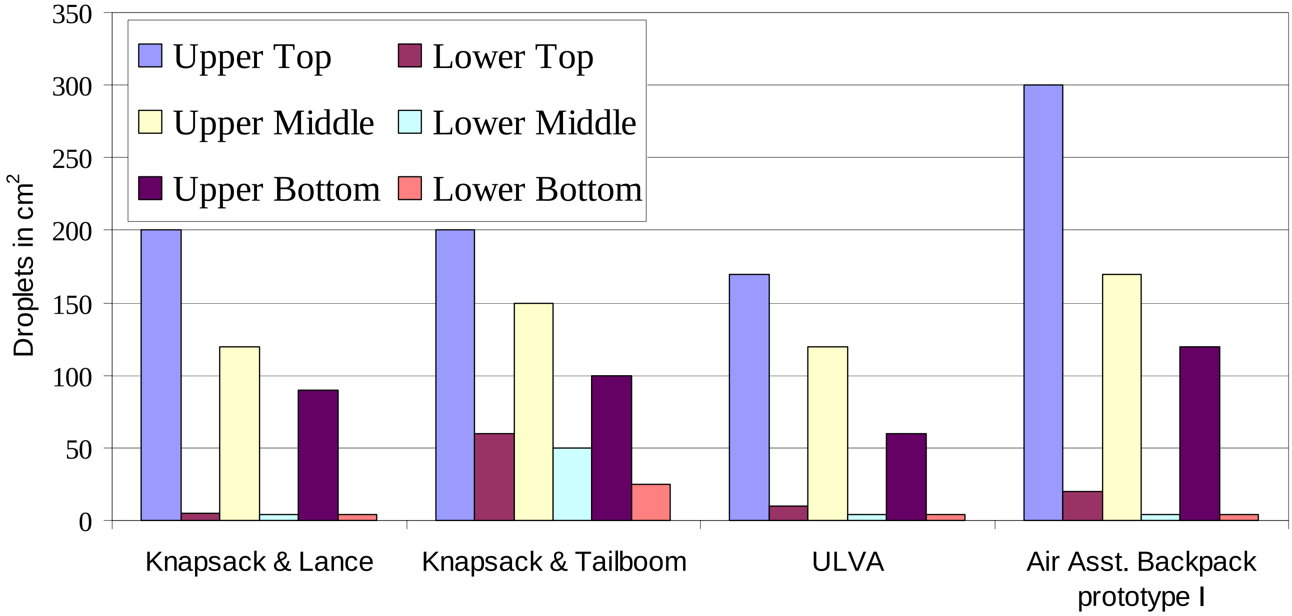
A commercial knapsack motor sprayer, also known as mist blower, (Arimitsu - Japan) was tested in addition to ULVA sprayer and a Lever operated Solo sprayer with X2 and X3 nozzles. The Arimitsu knapsack motor sprayer was modified and an extension hose was constructed to release the air-assisted spray few cm above the ground, in front of the operator. Technical data on the sprayer is given in table 1 [1999 report, p. 49]. Good results of field tests of spray deposited on water sensitive papers are shown in table 2 [1999 report, p. 49-50].

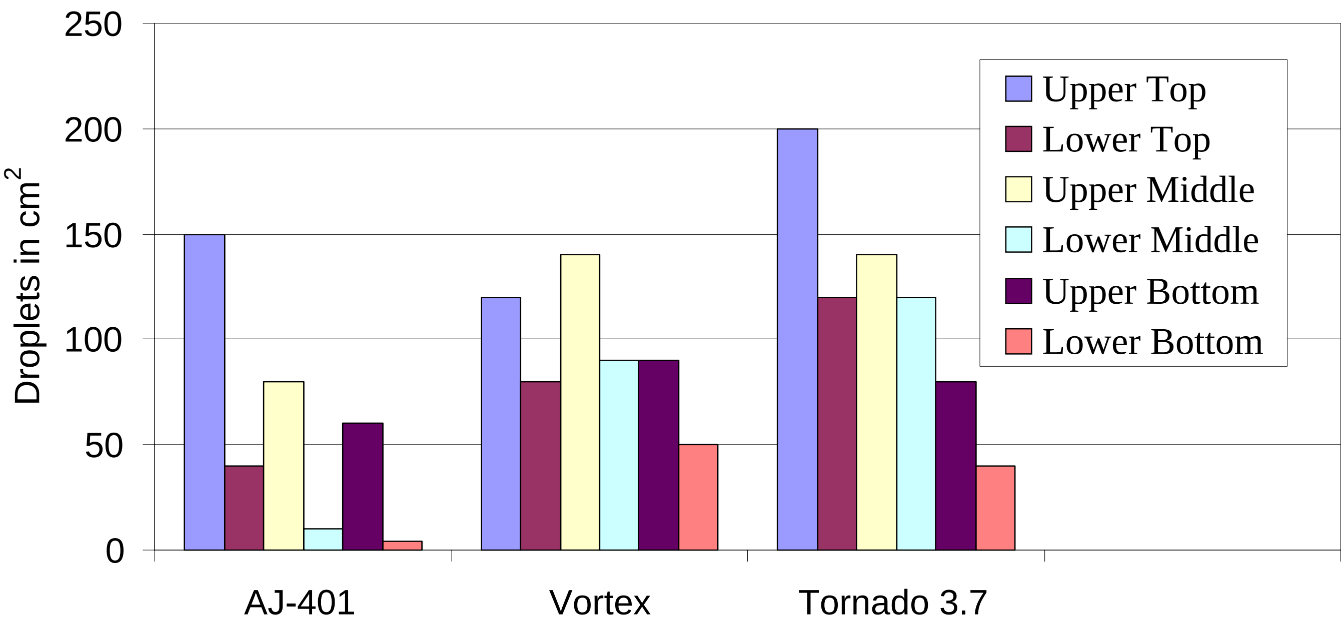
Qualitative and bioassay assessments were conducted with Kz-oil and Meiothrin insecticide at the recommended dosage with different pedestrian carried sprayers, through last four seasons. The spray volumes ranged between 13 to 166 l/ha against whitefly, on cotton, during the later parts of the seasons. According to the quality of spray coverage, the ground equipment could be arranged in descending order as follows: Knapsack motor sprayer Arimitsu with modified tail boom, ULVA sprayer, Lever operated Solo with X2 and Solo with X3. About 25 from droplets were deposited on the lower surface of the leaves of different levels of cotton plants with low-volume sprayers, ULVA sprayer and Solo sprayer with X2 nozzle. The optimum droplet size for controlling Bemisia tabaci ranged between 73 and 136 μm with average of 105 μm, as a suitable droplet size corresponding with the biological efficacy under the local conditions in Egypt [1999 report, p. 46].

2. Development of an under the canopy sprayer in Zimbabwe [1999 report, p. 42].

A knapsack-motorized sprayer was modified for underside application of the nontoxic pesticides in small farms. It did not provide considerable improvement over the good cover and control provided by the commercially available tail boom sprayer. The results of field tests of spray deposited on water sensitive papers are shown in figure 2a for pedestrian carried sprayer and figure 2b for tractor mounted sprayers.

Figure 2: The droplet density found on water sensitive papers for the knapsack and tractor mounted sprayers tested in Zimbabwe. The results for the drop-tube sprayer are for prototype No. I.





3. Sprayers developed in Israel

3.1. The Mark II prototype sprayer - The Tornado sprayer [1996 report, p. 133; 1997 report, p. 129; 1999 report, p. 45, 51].

The mark II drop-tube sprayer was developed with ground clearance of 60 cm to avoid hitting branches, which are crossing from one row to the next one (figure 3).

Figure 3: Mark II prototype sprayer (Tornado) with high ground clearance, mounted on a tractor.

From the height of 60 cm to the plant top, the drop-tubes were applying the air-assisted spray, similarly to the way it was done in the Mark I prototype. From the ground to the height of 60 cm a new technology was developed to provide air streams with a velocity component in the upward direction as shown in Figure 6. Air streams from two adjacent drop-tubes are aimed at the ground. When hitting the ground each air stream is split into two, each aiming at the hitting point of the air streams from the neighboring drop-tubes creating an upward current which carried spray droplets that were deposited on the lower sides of the leaves.

Figure 4 shows the cover density of the UV tracer application with the Tornado sprayer. Figure 5 shows the droplets density with this sprayer.

Figure 4: **The covered area found with UV tracer for the tractor mounted sprayers and for the Drop-tube knapsack sprayer (prototype No. II).**

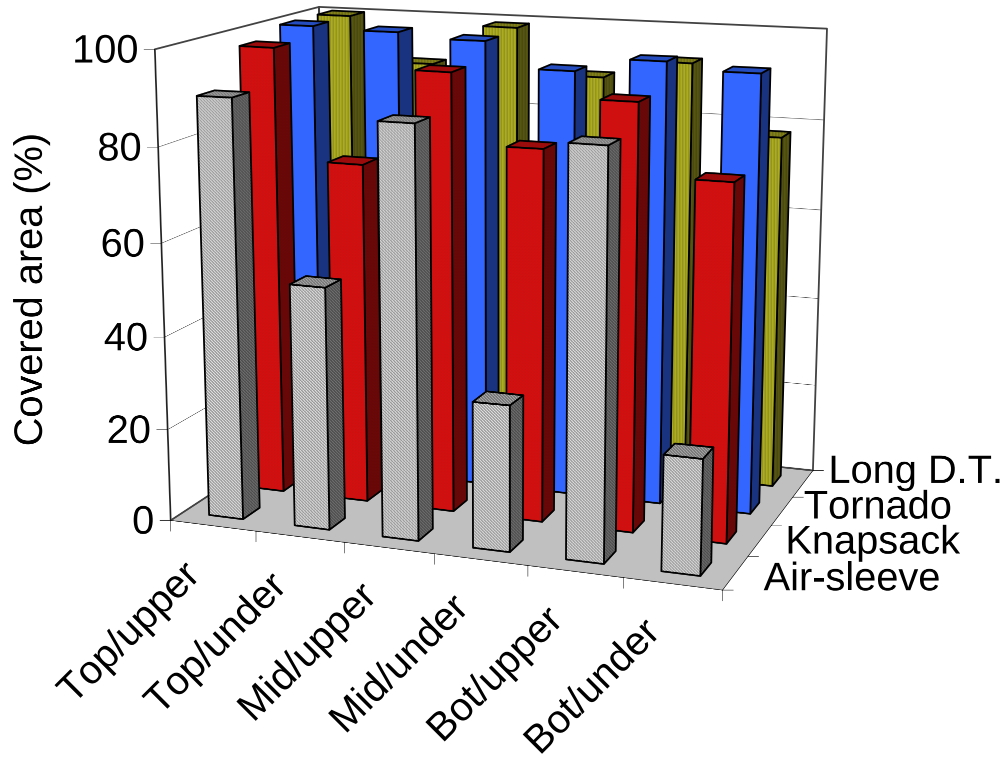
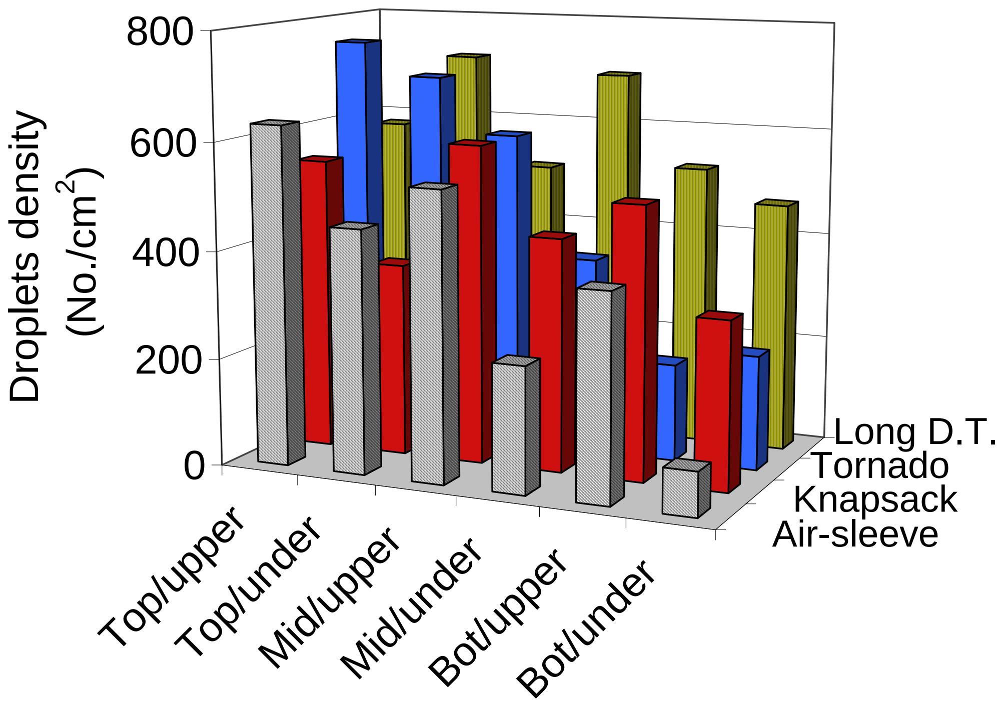


Figure 5: **The droplet density found with UV tracer for the tractor mounted and for the Drop-tube knapsack sprayer.**



Figures 4 and 5 also compare the results with other sprayers, including the Air-sleeve sprayer. To improve the deposition uniformity of the Air-sleeve sprayer a slower speed of 4.8 km/h and two application passages on each row were used. This increased the flow capacity to 500 liter per hectare, which is about 5 times above the commercially recommended amount. The cotton density was slightly lower and the conditions were better than the conditions for the Tornado test.

For reference figures 4 and 5 also show the covered area and droplets density for the Mark I sprayer. It is clear that the Mark I gives better coverage than the Tornado and the air-sleeve sprayers, and, as shown in project sections which dealt with field trials for whitefly control, it was better in controlling infestation in cotton [1998 report, p. 150].

3.2. The drop-tube knapsack sprayer

The drop-tube knapsack sprayer (prototype II) (Fig 6) employs four rotate discs (Ulva+ atomizers) to form the droplets.

Figure 6: The Drop-tube knapsack sprayer - prototype No. II.

A 12 Volts rechargeable battery is providing the power to the atomizers and to a blower mounted at the top of the sprayer. The blower blows the air through a perforated tube with many small outlets along the two sides. The streams form air curtains directed toward the row on both sides of the sprayer. The spray droplets created by the atomizers are released and mixed in the air curtains, which carry them until deposition on the cotton canopy. [1998 report, p. 122].

The sprayer is adjusted to deliver an average of 940 ml per minute or 56.4 liter per hour. A walking speed of 2 km/h delivers 282 liter per hectare (28.2 liter per dunam). When 16-liter active material per hectare is required 266 liter of water is necessary to dilute the active material. Figs 4 and 5 compare the application uniformity of the Backpack, Tornado and the Air-sleeve sprayers [1999 report, p. 42-45].

E. Conclusions

Two tractor-mounted and three knapsack cotton sprayers were developed and tested. Field tests showed higher uniformity of spray deposition for all of the newly developed sprayers as compared with commercially available machines. Efficacy tests are presented in the reports of the field trials group [1999 report, p. 10-21].

PART 3: BIOLOGICAL CONTROL

Chapter 4: *Bemisia* biology and natural enemies

A. Goal

To understand the dynamics of the pest and its natural enemies in order to facilitate optimal pest management.

B. Questions asked within the goal:

To determine the following:

1. Pest biology and dynamics
2. Natural enemy identity, biology, dynamics and impact - how important are they, and when are they important?

   Parasitoids

   Predators

1. Insecticide use
   a. Effects of treatments on natural enemies – is it damaging to control, can it be improved?
      i. Commercially used materials
      ii. Our preparations

   b. Possibilities of insecticide integration
2. Impact of neighboring crops – do they help or damage management of *B. tabaci* populations?

C. Experiments conducted to answer each question

1. Pest biology and dynamics

In each country, weekly or fortnightly counts were conducted to determine the levels of infestation by whiteflies. The counting method was uniform (following training of the personnel) and consisted of picking 20-50 leaves, each from a separate plant from the more infested levels of the plants (leaf 5-7 from the top). The whitefly nymphs that have reached, at least the third instars on each leaf were counted. This method of producing an estimate of whitefly population levels had several merits. Relative ease of counting the largest stages of the pest; relating only to whitefly levels that could have been parasitized, and ignoring earlier stages thus avoiding counting the same whitefly twice week after week (once as a first or second instar and later as a fourth instar).

2. Natural enemy identity, biology, dynamics and impact

   a. Parasitoids

      Parasitoids were counted with the count of whitefly nymph observing parasitism through the transparent whitefly nymphal skin. Alternatively, parasitoids were allowed to emerge from their pupae and counted as adults (Egypt).

   b. Predators

      As said, parasitoid numbers were estimated with those of their hosts; predators were counted, at the same time as their hosts, as follows: in each field, 6, 1-meter sections were selected at random. In each, the plants were visually scanned and the numbers of predators (insects and spiders) that were observed was registered. Whenever predaceous mites were sampled, they counted on plant leaves that harbored the highest number of *B. tabaci* nymphs.

3. Insecticide use

   a. Effects of treatments on natural enemies

      i. Commercially used materials

      ii. Our preparations

   b. Possibilities of insecticide integration

   The effects of commercially used insecticides were estimated by making the same counts in treated and untreated fields to estimate the effects of treatments on the counted insects. Our preparations, materials number 4, III and the oils were not available in commercial quantities or preparations. Consequently, they were hand-sprayed with a backpack sprayer following a count that showed the presence of the natural enemies in question. Counts were conducted 2-10 days after the treatment to assess the results.

   Insecticide integration was examined by looking at the effects of commercial treatment on natural enemies. It was also tested specifically by treating pre-infested and pre-parasitized sections with specific insecticides.

4. Impact of neighboring crops

   Neighboring crops can be a boon or a detriment to cotton; depending on which insects they cause to migrate into the cotton field. Two cases were examined, corn, a plant known to harbor predators attracted by the abundant pollen (e.g. *Orius* spp.) and Sunflowers, that in Israel are known to occasionally harbor very large whitefly populations.

   A Cornfield was grown adjacent to our cotton and counts were made of the predators in it and in the adjacent, untreated cotton rows.

Levels of whiteflies and predators were estimated on sunflowers and their migration rates into a cotton field were studied by trapping.

D. Results and conclusions

1. Pest biology and dynamics

   Whiteflies were found in all countries throughout their season, and two patterns were observed. In most cases populations were lowest when cotton was young and rose with the season, a sharp rise usually occurred in late July or August. Maximal levels in untreated fields were as low as 2.5/leaf [1997 report, p.194] but reached high levels like 68/leaf [1998 report, p. 162-165]. In Ethiopia, high populations were observed early in the season due to their development on alternative hosts from which they moved-on to cotton [1997 report, p.221]. Very different levels could be found in the same year in two locations, e.g. 68/leaf in Kafr El Sheikh, and 3.5/leaf in El Minia both in 1998 [1998 report, p.200, 203]. Population fluctuations in the field showed several peaks; however, those did not coincide with life cycles and did not facilitate calculating the numbers of generations. Peak populations were observed in July [1995 report, p.92], August [1998 report, p.161 –165 & 199] and September [1998 report, p.198].

2. Natural enemy biology, dynamics and impact

   a. Parasitoids

      In Egypt and Israel *Encarsia lutea* Masi and *Eretmocerus mundus* (Mercet) were the predominant whitefly parasitoids [1995 report, p.101]. In Ethiopia and Zimbabwe *Encarsia transvena* and *Eretmocerus mundus* or a very similar species, were found. Rates of parasitization often rose with the season. They reached various levels up to 90% [1995 report, p.92; 1998 report, p.199] and the 4-year averages for Israel and Egypt were between 65-70%. For Ethiopia, the one reported year averaged 47% parasitism. No correlation was found between % parasitism and whitefly population levels (4-year coefficient of correlation for Egypt 0.48 and for Israel -0.2). The use of insecticides often did not affect % parasitism [1998 report, p.199]. However parasitism was significantly reduced, in some cases [1997 report, p.151–158]. Apparently Acetamiprid is one of the more harmful insecticides to the parasitoids [1998 report, p.158, 161].

      The high percentages of parasitism indicate that even when whitefly populations were very high and reached damaging proportions, the rate of whitefly reduction was significant. Remembering that each parasitoid means one dead whitefly, and that the sex ratio is about 50% female whiteflies, it is easy to calculate that parasitism alone prevents a very significant increase of whitefly populations. This is especially significant in the light of quick resistance buildup in whiteflies – resulting from the need to treat with insecticides.

b. Predators

Many predator species were found. Most coincided among the locations but some did not [e.g. compare lists in 1996 report, p.145 with predators mentioned in 1996 report, p.168, 169; 1998 report, p.157 and 176]. The more generally occurring ones are *Chrysoperla carnea*, *Orius* spp. *Deraeocoris pallens* (In Israel), *Campilomma* spp., *Coccinella* spp., *Hippodamia variagata*, *Scymnus* spp., spiders of various kinds and predaceous mites, esp. *Amblyseius swirskii* (studied only in Egypt). Since none of these is specific to whiteflies, and some are probably not regular predators of these pests, there was need to establish methods to evaluate their value in our context. This was done by drawing correlation between their abundance and that of whiteflies, and by conducting specific observations on their behavior. The only predators showing overall correlation with the populations of whiteflies were species of *Orius* (corr. index = 0.78). The most abundant predator species was *C. carnea* that also was found throughout the season, whereas some of the others were present especially in the earlier part. Studies conducted with *A. swirskii* in Egypt also indicate that the abundance of this species may be linked with that of the whitefly [1996 report, p.149]. Direct observation of predators on plants in relation to whiteflies showed that predators frequent whitefly infested leaves [1995 report, p.98, 99; 1997 p.158-160; 1998 p.140-165]. This relationship is not always simple, but the Heteroptera, and probably also the mites, seem to be important controlling factors of whitefly populations.

3. Insecticides use

a. Effects of treatments on natural enemies

Commercially used materials;

Generally we found that predators were more sensitive to insecticides than were parasitoids [1999 report, p.71-72]. Again, like in the case of parasitoids, Acetamiprid proved to be detrimental to the natural enemies, with the exception of the predacious mite *A. swirskii* in Egypt [1999 report, p.86]. Non-conventional insecticides, *Beauveria bassiana*, jojoba oil, mineral oil, and the IGRs: Diafenthiuron, Pymetrozine, and pyriproxyfen were tested against the whitefly and natural enemies, predators as well as parasitoids. In contrast to conventional materials, most of these insecticides did very little damage to enemy populations while killing high proportions of the pest [1997 report, p.195-199]. Similar results were obtained with Cascade (Flufenoxuron) Kemesol (summer mineral oil) and

National (winter mineral oil) [1998 report, p. 204]. The same trend was noticed by using Natural (vegetable) oil, Bemistop and Buprofezin [1999 report, p 71].

Tests were also run to see which of certain insecticides, usually used in the cotton field would be the least harmful to parasitoids. Here, again the material Acetamiprid proved to be harmful to the parasitoids. It is recommended that these tests be continued for more materials and under more varied field conditions.

b. Our preparations:

Materials number 4 and III that were developed during the course of the project were tested as to their effects on natural enemies in the field. This was usually done by selecting fields that abounded in natural enemies and treating them with the material to be tested.

Material 4 was relatively innocuous [1997 report p.157-158]. Material III showed detrimental activity in the lab and in the field. In the latter case, the damage to natural enemies seemed short-lived [1998 report, p.163].

4. Possibilities of insecticide integration:

Integration at large will be discussed in a different chapter. Here we'll only mention the fact that various materials found less harmful to natural enemies and yet being able to control the whiteflies, can serve as important tools for the control of the pest, especially early in the season [1997 report, p.252].

5. Impact of neighboring crops.

We did not find any advantage in growing corn near the cotton. The main predators therein, *Orius* spp. did not seem to migrate into the cotton [1995 report, p.88]. Sunflowers harbored few natural enemies, and at times, a lot of whiteflies. Thus, at best, the proximity of sunflowers will not cause a whitefly outbreak, but we should not rely on this crop as a refuge for natural enemies [1997 report, p.157 – 158].

PART D: ESTABLISHMENT OF ECONOMIC THRESHOLD LEVELS

Chapter 5: thresholds for whiteflies and aphids

This study was mainly conducted with whitefly since aphids are not considered a major pest in Israel and Egypt. In Ethiopia, in the trial plots for aphids, there were generally low levels of the pest [1997 report, Chapter 15; 1998 report, Chapter 19]. This phenomenon also occurred in trials that were scheduled to be sprayed according to threshold level and also in trials in which the treatment was to be given according to the various stages of cotton plant development. In a single trial [1998 report, p. 255] yield was decreased by 55% in the untreated control in comparison with the treated control. In Zimbabwe in 1999, 2 new oils were tested against the standard insecticides and controls and here also the infestation level was lower than the standard threshold level and there was no effect on yield. For these reasons, this report will concentrate mainly on whitefly.

1st. Overall Goal

To develop an economic threshold level for whitefly.

We define a threshold level as the point at which the cost of treatment is equal to, or less than, the cost of the anticipated monetary loss resulting from reduced cotton yield or lint quality if the treatment were not applied.

The cost of the treatment is known, but estimation of the anticipated damage is practically impossible. Whitefly may cause significant reduction to yield by reducing the number of bolls and their weight. It may also damage lint quality (micronaire, length, etc.), increase the sugar content in the fibers and cause stickiness or gray color [1997 report, chapter 13].

Development of the pest in the cotton fields increases with the season. Terminating cotton varieties transfer all of the photosynthesis to the fruiting bodies in the late growing stages (the plant enters “cut-out” and vegetative growth ceases). Reduction of photosynthesis caused through whitefly damage, directly effects the yield quantity and or quality with no possibility for even partial compensation by the plant unless the season is particularly long.

2nd. Questions asked within the goal

1. How may the expected damage caused by different population levels be estimated?
2. What is the effect of intervention (i.e. treatment) on the development of the target pest during the rest of the season?
3. What is the connection between the threshold level to the insecticide used?

C. Experiments conducted to answer these questions

See:

Israel: 1995 report, pp 118-135. 1996 report, pp 180-189; 1997 report, pp 226-243; 1998 report, pp 221-228 and 1999 report, pp 103-114.

Egypt: 1995 report, pp 136-138; 1996 report, pp 190-209; 1997 report, pp 244-266 and 1998 report, pp 229-246.

Ethiopia: 1997 report, pp 275-278 and 1998 report, pp 247-252.

D. Results

1. Sub-Goal 1

Estimation of the population size that accompanies the damage threshold.

Eight field trials were conducted from 1995 to 1998, isolating 30 different populations of different sizes and distributions throughout the seasons were isolated [1999 report, p.104, Table 14].

Populations were calculated using the average number of immatures on the maximally infested leaf during the last 60 days before first defoliation treatment. For this purpose, the immatures were counted twice a week for the entire period. Using linear interpolations, the daily values were calculated for the days between counts. The total value for the 60 days was divided by the number of days to calculate the average daily population. In the Table 15 [1999 report, Chapter 16], the populations were arranged according to ascending order while monitoring their effects on yield and quality components. From this table it is possible to estimate the damage threshold as being close to the average of 20 larvae and pupae.

The population development rates differ. Some remain low to moderate whereas others increase to form heavy infestation at boll opening time. The latter cause damage due to fiber discoloration estimated at 15% of fiber value and warrant chemical treatment.

In our calculation method, we always used the lowest occurring population of about 5 nymphs/leaf as a base line for comparison, rather than a zero population. This prevents us from pinpointing the exact level (of ca. 15-20 nymphs/leaf) at which the threshold is attained.

2. Sub-Goal 2

How does the spray treatment effect the future development of the population?

Building a whitefly population within an unsprayed cotton field depends on:

One. Migration into the field.

Two. Rate of adult oviposition.

Three. Survival of various stages.

Our study was conducted in the coastal plain of Israel where most of the whitefly populations result from initial early season infestations. The fields are heavily treated against pests other than whitefly, a fact that greatly reduce the activity of natural enemies. In the 1999 Maayan Zvi trial, a single treatment of Diafenthiuron applied to a low level of nymphs (on 23.6.99) reduced the population by approximately 45% in the 3 combinations tested [1999 report, p 112, Table 18].

This population reduction lowered the rate of reproduction resulting in a smaller population throughout the counting period [1999 report, pp. 111-112, Figures 7 and 8].

Thus, a threshold may be reached in two ways: 1) to spray when the population reaches the threshold level, reducing it through the treatment. 2) to treat early treatment and reduce the population in its initial stages. The smaller population will reach the threshold at a later date while reducing the cumulative damage throughout this period.

This policy will avoid the outbreaks of populations at the end of the season.

3. Sub-goal 3

A summary of insecticidal activity is shown in Table 1.

Generally the different treatments were composed of a series of 1 to 4 sprays, labeled "control strategy".

The calculations in the table were arranged as follows:

1. Average populations for each treatment throughout the counting season were calculated in each trial.
2. The percent of the average population for the treatment was calculated from the average population of the untreated control, in each within the treatments of the trial.
3. The percents of nymphs calculated was deducted from 100%, giving the percent of the population reduction in the said treatment in relation to the corresponding control.

Table 1: Decreased population (season average) in percent from the corresponding untreated control

Ave. nymphs/maximal infested leaf, in treatment/location:	Tzora Acala	Gvir	Maayan Zvi Acala	Ein Shemer	Maagan Michael 97	Maayan Zvi 99	M. Michael 98	Maanit
Untreated control	22	22	37	39	100	124	180	363
Aldicarb * 1 application				80				80
Pyriproxyfen * 1 application	44	60						
Diafenthiuron – 2 applications			91		82	86	86	
Material 4 * 2 applications Tornado					6		20	
Material III * 2 applications Tornado			64				47	
Material III * 4 applications Sleeve			75			73		
Material III * 4 applications Tornado			78			69		

Estimations concerning reduction of the whitefly populations following from various control strategies in comparison with the untreated control may be seen in Table 2.

The first spray application of each strategy in this Table is at the time that the nymph count on the maximal infested leaf was 6-10.

Table 2: Estimation of whitefly nymph population reduction (seasonal averages) for various control strategies

Strategy		Reduction percent
Chemical	No. sprays	
Diafenthiuron	2	86
Aldicarb	1	80
Material III (weekly) Sleeve or Tornado	4	74
Material III (bi-weekly) Tornado	2	55
Pyriproxyfen	1	52
Material 4 (bi-weekly) Tornado	2	13

The threshold level will be dependent upon the chemical to be used or else the population rate will determine the choice of chemical. A similar approach may be found in 1997 report, p. 244 for Egypt.

E. Discussion

The nymph stage is the most effective for control nowadays as most of the best chemicals in use against the target pest (Buprofezin, Acetamiprid and Diafenthiuron) controls the nymphal stages. That is why the threshold level in this study was determined by nymph counts.

Whitefly damage is cumulative over a period of time and cannot be treated as a fixed threshold level, but as an average throughout time that should not be exceeded. If the aim is not to exceed the average of 15-20 nymphs for the period of 60 days before 80% of the bolls are open in the field, it should be spoken of in terms of "control strategy" and not of a specific threshold. There are different strategies that attain the same goal and a way must be found to recognize in real time the most effective strategy in terms of control cost, prevention of developing resistance, protection of beneficials, etc.

The conclusions of this work should be categorized according to the conditions in which they were tested:

High yield of 5-6 tons/ha.

Terminating varieties with limited season.

There are no beneficials in the field as a result of the treatment for other pests.

Adult migration into the field limited to the first 90 days and later reproduction within the field.

At dates of treatment for whitefly, adults, eggs, pupae and larvae were present in the field.

Under the above conditions, the treatment according to the threshold level of 15-20 immatures/leaf with chemical that controls nymphs will ensure keeping the average population to less than the preferred threshold and will prevent damage to yield and lint quality. This policy will be economical, as the first damage that appears at a level of more than 15 immatures/leaf is the gray color of the fibers. With yields of 5-6 tons/ha, this is equal to the cost of 6 treatments with the most expensive chemicals.

The supposition is that if the threshold is based on the number of nymph, the treatment will be given using a chemical that controls larvae. In the field where there are a variety of adults, eggs, nymphs, and pupae, there is the danger that the stages that are not controlled will be the cause of a great number of treatments. Such an occurrence was found in the Hahotrim trial in 1996, Maagan Michael trial in 1998 and Maayan Zvi trial in 1999. In these trials, the treatments applied at the end of the season did not succeed in reducing the populations.

Gerling etc. [1997 report, Chapter 9, Figures A6 and B6] indicate the great variability among the different fields. In the Revadim trial (A6) from a large population of adults, a low population of nymphs developed, while in the Maayan Zvi trial (B6) from a much smaller population of adults, a larger population of nymphs evolved.

The 1999 Maayan Zvi trial indicates that the early treatment on a much lower level than the threshold is able to decrease the average population size by 45%. This is caused by the fact that the absolute population level throughout time is the function of the original level and of the reproduction rate. In a field in where there is no activity of beneficials, a spray treatment will reduce the initial level without effecting the reproduction rate.

In a situation where effective natural enemies exist in the field, one should look for selective insecticides, which will not destroy the beneficial populations.

From this it seems that the early treatment may have an advantage over the treatment according to thresholds: The cumulative damage is avoided in the period between the early treatment and the time that the threshold level would have been achieved if the treatment had not been applied.

In addition: an early decrease in population may prevent later population outbreaks.

Along with this advantage of early treatments there are also disadvantages. The first is that if it later proves that pest reproduction in the field is slow (as in the Revadim trial in 1997) the treatment could have been avoided. A second disadvantage is in fields that attract adult migration from surrounding fields. Treatment according to threshold level would successfully control the newly acquitted nymphs, while early treatment would not have controlled them.

This study determines the average level through time above which damage would be incurred. Therefore, treatments according to this threshold level will prevent it. But it does not provide a solution to the "optimal strategy" according to dates of spray, the chemical that should be used, the final number of necessary treatments and their cost. However, it does indicate the complexity of the problem. Although it appears as if treatments applied according to the threshold of 15-20 nymphs will prevent damage and will be economical, we don't know the optimal time for treating or if the early treatment will really save money and improve control each time.

F. Conclusions

In fields in with low whitefly reproduction (migration and development within the field) there will be a low population and a threshold level of 15-20 larvae and pupae may be used.

In fields with high whitefly reproduction rates one should speak in terms of "control strategy" and not in terms of threshold. The aim of the strategy would be to ensure that the average population would not rise above 15-20 nymphs/maximum leaf as an average for the entire season. The strategy would include a series of treatments (date and chemical) at varying thresholds that would bring about these results at a minimal costs while retaining the proper policy for preventing resistance of the pest for the various chemicals. In order to achieve better decisions for preparing these strategies, this study should be continued in this direction.

TECHNICAL APPENDIX

1. Guidelines for preparation of oil-based target-oriented pesticides

Dan Veierov, Annie Fenigstein, Miriam Eliyahu, Nadav Aharonson,

A. Introduction

Vegetable oils (VO) are heterogeneous mixtures whose compositions vary between and within kinds, between manufacturers and even between batches. Moreover, the bioactivities of VO are probably take effect by contact; therefor their performance is strongly influenced by their formulation/application mode. Therefore, a laboratory setup in the field is required for development and for quality assurance of VO as pest control agents. The following guidelines offer a simple setup as a model for development and preparation of formulations based on oils available in the target countries. They include recommendations for a general development scheme (1) as well as detailed procedures for preparation (2) and evaluation (3-5) of VO emulsions.

B. A general development scheme:

The development scheme is divided into four subsequent developmental stages, according to the formulation types and the associated assays for their control-related properties.

STAGE	FORMULATION STUDIES	ASSAYS
1- Simple emulsions	VO characterization and screening of primary emulsifiers	Activity profiles Phytotoxicity tests
2- Experimental formulations	Process variables and Composition variables	Stability Sprayability Activity profiles Phytotoxicity tests
3- Scaling up	Process variables	Abbreviated quality tests (in the field)
4- Optimization and long-term stability	Process variables	Abbreviated quality tests (in the field)

Remarks:

Stage 1 is aimed at the selection of the primary emulsifier (types and concentration range) as well as at the characterization of VO by their bioactivities and phytotoxic trends. This stage requires small amounts of simple emulsions consisting only of VO, emulsifiers and water.

Stage 2 is aimed at the assessment of the influence of various formulation variables on performance-related properties of VO. Small-scaled preparations are adequate do at this stage.

Stage 3 consists of scaling up the most promising formulations, mainly by adjustment of process variables. The scaled up formulations are then subjected to primary field tests. Each preparation batch should undergo shortened quality tests prior to its application in field.

Stage 4 includes further optimization, as required following field trials as well as long-term stability studies.

C. Preparation:

The following table presents the composition of two types of recommended VO emulsion concentrates. One emulsion variant (a) is prepared in the absence of any auxiliary ingredients ("optimized VO emulsions"). Optimized emulsions show good and consistent performance but their preparation on a large scale might require comparatively expensive equipment. The second emulsion variant (b) ("stabilized VO emulsions") contains cetyl alcohol as stabilizer. It is highly stabilized, and shows good resistance to aging effects.

The emulsion composition compositions are:

	OPTIMIZED EMULSIONS	STABILIZED EMULSIONS
(a) Oil phase		
Cetyl alcohol	----	6 w
Tween 80	1 w	3 w
Vegetable oil	99 w	91 w
(b) Water phase		
Deionized water	228 w	197 w
Tween 80	2 w	3 w

The cetyl alcohol purity grade is $\geq$ 96% (m.p. 48-50). Tween 80 (polyoxyethylene [20] sorbitan monooleate) is a syrup having a number-average molecular weight of 1.31. The vegetable oils are of food or pharmaceutical grade and can be employed without further purification. The oil and the water phases should each be prepared separately by heating its components at 55-58^{o}C with magnetic stirring until solution is complete.

Emulsions are produced in 100-200 g lots for laboratory studies, and in 4 -10 kg lots for field experiments employing a rotor-stator type homogenizer, (e.g. Polytron PT 3000 Kinematica AG). Dispersion tools ("aggregates") are selected to exert maximal shear for a given lot size, e.g. 7-mm and 20-mm diameter aggregates of small- and large-scale preparations, respectively. The emulsion-vessel dimension should be of the smallest diameter possible to permit the aggregate to be immersed 2/3 below the surface and 1/3 above the bottom of the vessel, at all stages of the preparation.

The oil phase is placed in the emulsification vessel and mixed at moderate speed (5000 rpm) while being heated to 80-85^{o}C. Homogenization speed is then raised to 25,000-28,000 and the pre-heated water phase (85-87 ^{o}C) is added dropwise with the aid of a separator funnel into the oil phase, till phase inversion is observed. After completion of phase inversion, the rest of the water phase is added at a relatively higher rate, and homogenization is continued for at least more 5 minutes (usually the adequate period for the stabilized types) up to 15 minutes (sometimes needed for optimized types) according to the stability of the resulting emulsion.

The reaction vessel is then removed and allowed to be cool rapidly (cooling rate of 5^{o}-10^{o}C/min) to 40^{o}C, and made up to weight. The fresh optimized oil emulsions are transferred to lid-covered cylindrical containers (20 cm diameter) equipped with a 4mm bore faucet. The emulsion is kept in the dark for 48 hr, and is then permitted to flow without mixing through the faucet into a storage tank. The upper layer (10-15%) of the emulsion is collected, to be added as the water phase in subsequent preparations, or discarded.

D. Stability assessment:

<u>For primary assessments</u>: Emulsion stability should be evaluated using the modified technique of Manthey (1992). Samples of the appropriate size should be transferred to graduated test tubes and shaked vigorously for 10 seconds at ambient temperature. Sample size and test tube dimensions should be selected according to the oil phase concentration to allow its accurate determination. Emulsion stability is evaluated visually at various intervals after shaking on a scale of 0 to 100, where: 10 = oil layer, water phase clear (no emulsion present) ;20 = oil layer, water phase slightly opaque; 40=oil layer, water phase more opaque; 60 = thick creamy layer, water phase opaque; 80=thin creamy layer, water phase opaque; 100 = no creamy layer, water phase opaque (uniform emulsion). Each treatment should be replicated three times.

For relatively stable emulsions: The same procedure should be used to assess the Stability Index which is defined as (100 - the percentage of the column occupied by the developing layer) multiplied by the turbidity of the main body of the emulsion relative to its value immediately after shaking. Each measurement has to be replicated three times. Aging effects on emulsion stability can be determined with the same procedure, when the samples are taken after shaking the concentrated emulsions that had been stored in the dark at ambient temperature.

E. Activities on the target-pest:

Residual bioactivity and biopersistence: The various toxicological and behavioral aspects of the formulations should be evaluated by comparative laboratory bioassays: residual toxicity, settling deterrence and oviposition deterrence. In these assays, the target should be exposed to the foliar residues of the chemicals at various aging intervals. For assays conducted with immatures, the residual toxicity meant toxicity of the chemical residues to immature stages after the activity as an oviposition deterrent had been reduced or had ceased. The laboratory bioassays should be carried out in leaf cages under no-choice conditions.

Bemisia has to be reared on cotton plants in a greenhouse. Female whiteflies have to be caught with an aspirator and transferred to clip-on leaf cages (transparent cylinders, diameter 2 cm, and height 7 cm). The leaf cages, each containing a known number of females (20-40), should be attached to the lower sides of pre-treated potted cotton seedlings. The plants should be kept for various aging intervals under controlled conditions. Settling and mortality of adults should be recorded after exposure of 2, 4-5 and 24-27 h; and eggs after exposures of 24-27 h. Mortality results should be corrected according to Abbott's formula. Settling deterrence has to be expressed as a Settling Ratio (SR_t = T/C, where T and C are the percentages of adults settled on treatment and control, respectively, after t hours exposure).

All experiments should be replicated six times.

Spray toxicity: *Bemisia* rearing should be execute as mentioned above. The seedlings with the pest should be held at room temperature with average minimum and maximum temperatures of 24 and 28^{o}C. After 24 h the females should be removed and the eggs on each leaf has to be counted. One can determine development of various life stages with the using a binocular microscope or a magnifying glass. Spraying of the various developmental stages should be carried out under a hood and then return the plants to the controlled-temperature room.

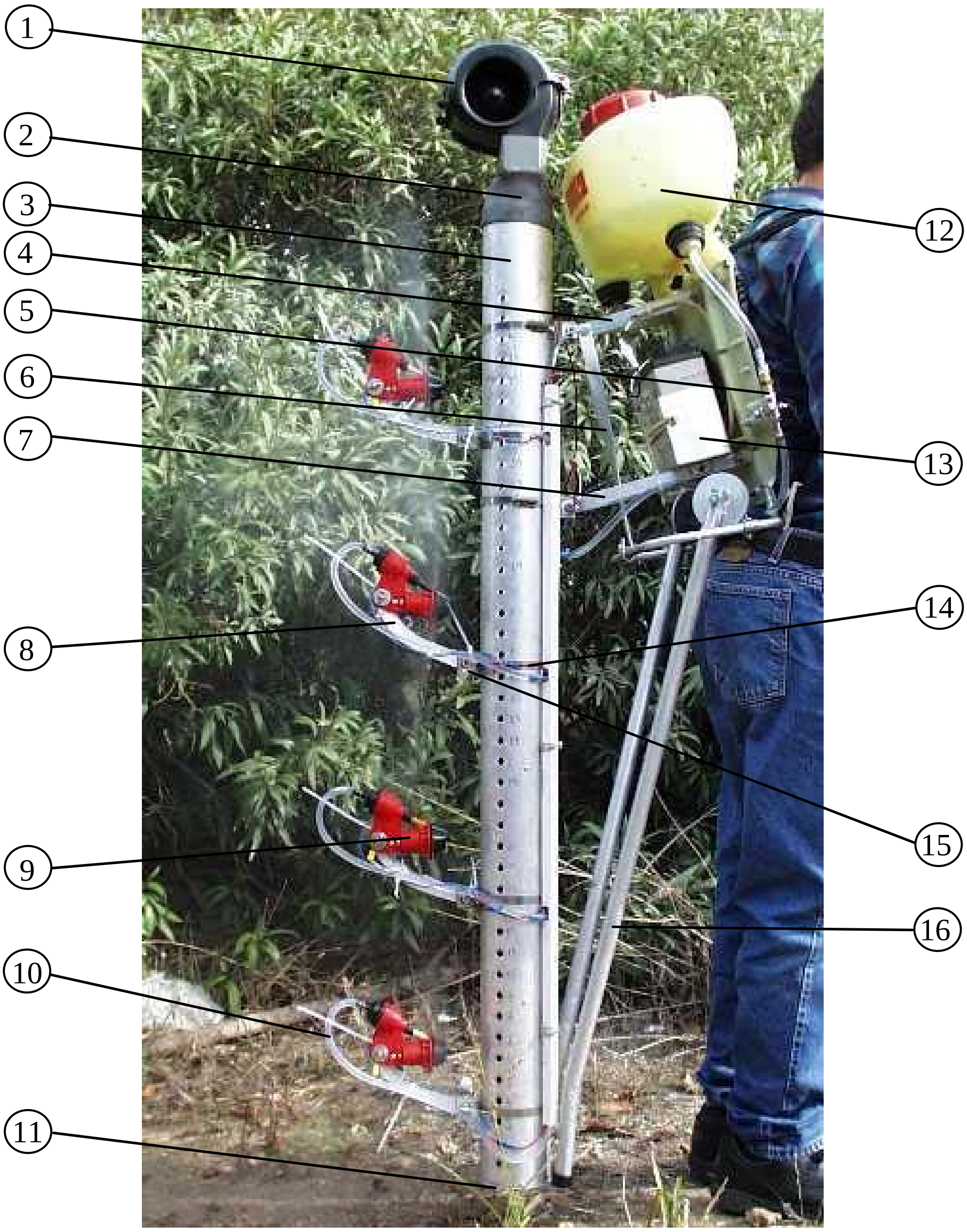
Percentage survival for each developmental stage should be determined according to the number of live pupae and empty puparia per egg.

F. Phytotoxicity tests:

The phytotoxicity tendency of surfactant solutions and oil emulsions should be evaluated by whole-plant bioassays. The emulsions has to be sprayed on potted young cotton and tomato seedlings at the four-leaf stage. All seedlings are greenhouse-grown, with one (tomato) to three plants (cotton) per pot. Plants should be surface-watered daily. The treated plants should be held under room conditions with average minimum and maximum temperatures of 26 and 28^{o}C, respectively, and photo-period of 16 hr. Relative phytotoxicity should be assessed by determinations of leaf injury and stem damage at several intervals post-spray. The percentage increment in the *number of nodes*, and in the *seedlings height* has to be calculated for each seedling individually, and than averaged for each treatment-date. *Leaf injury* should be assessed visually. Leaves should be assigned to one of six response categories: <1%, 1-10%, 11-25%, 26-50%, 50-75%, and >75% leaf damage. A rating for the treatment should be determined by averaging the samples, each of which consisted of ten potted plants. Sprays of deionized water served as the control treatment. Aging effects on the phytotoxixcity of the VO emulsions should be measured by taking samples from concentrated emulsions, shaking them and storring them for known interavals in the dark at ambient temperature before spraying.

2. Air assisted knapsack drop-tube sprayer parts & assembly description

Knapsack drop-tube sprayer - general and detail drawings



List of parts

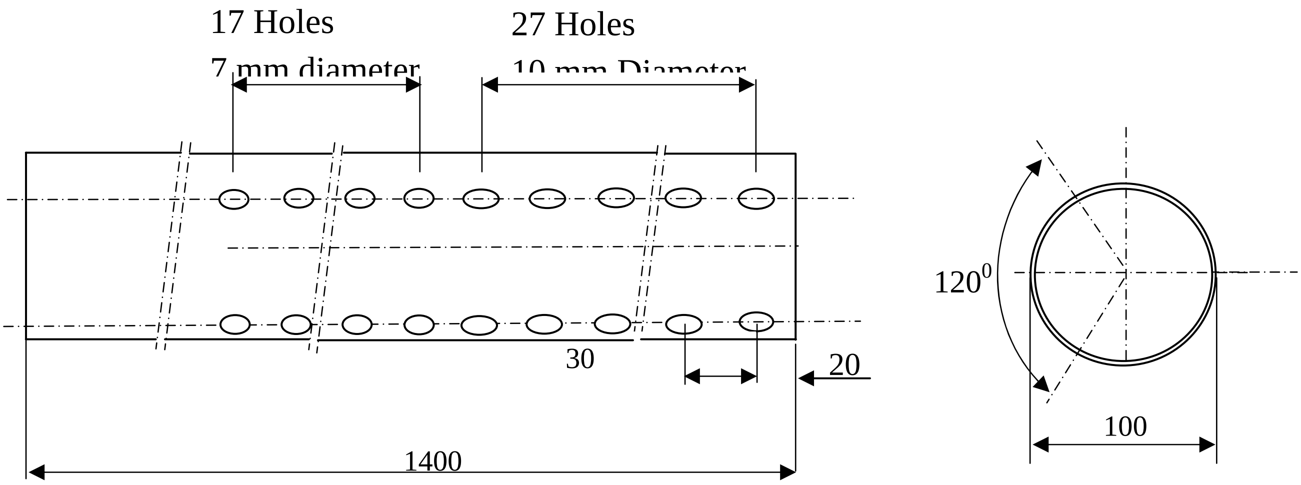
Part No.	Part description	Quantity	Materials & Suppliers
1	Blower	1	Part no. 0130063804 Bosch Co. Germany
2	Tube adapter	1	To be designed to fit the blower
3	Drop-tube	1	See detailed drawing
4	Support bar	2	See detailed drawing
5	Tap	1	3/8" 0n/off water tap
6	Support bar	1	See detailed drawing
7	Support bar	2	See detailed drawing
8	Support arc	4	See detailed drawing
9	Spinning disk	4	Part No. ULP/108 Micron Ulva Plus Micron Sprayers Ltd. UK
10	Connecting tube	8	6 mm ID flexible tube
11	Tube plug	1	See detailed drawing
12	Spray tank & backpack bracket	1	Solo Co. Ltd. Germany
13	Battery	1	12V, 17Ah Rechargeable battery.
14	Band	6	For 4" tube
15	Band connector	6	See detailed drawing
16	Folding leg	2	See detailed drawing

Note:
All measurements are given in mm unless specified otherwise.

Part no. 3

Quantity: 1

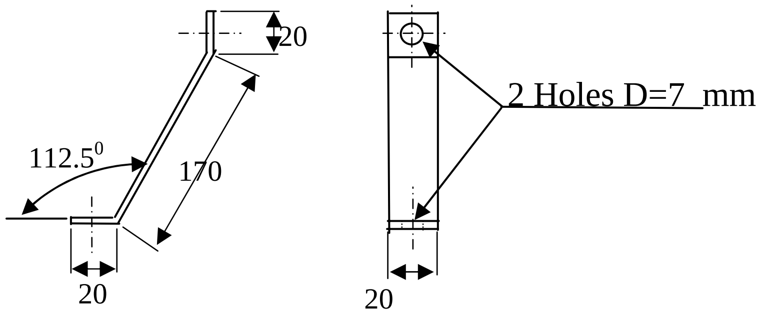
Material: Aluminum tube 100 OD x1.5



Part no.   4

Quantity: 2

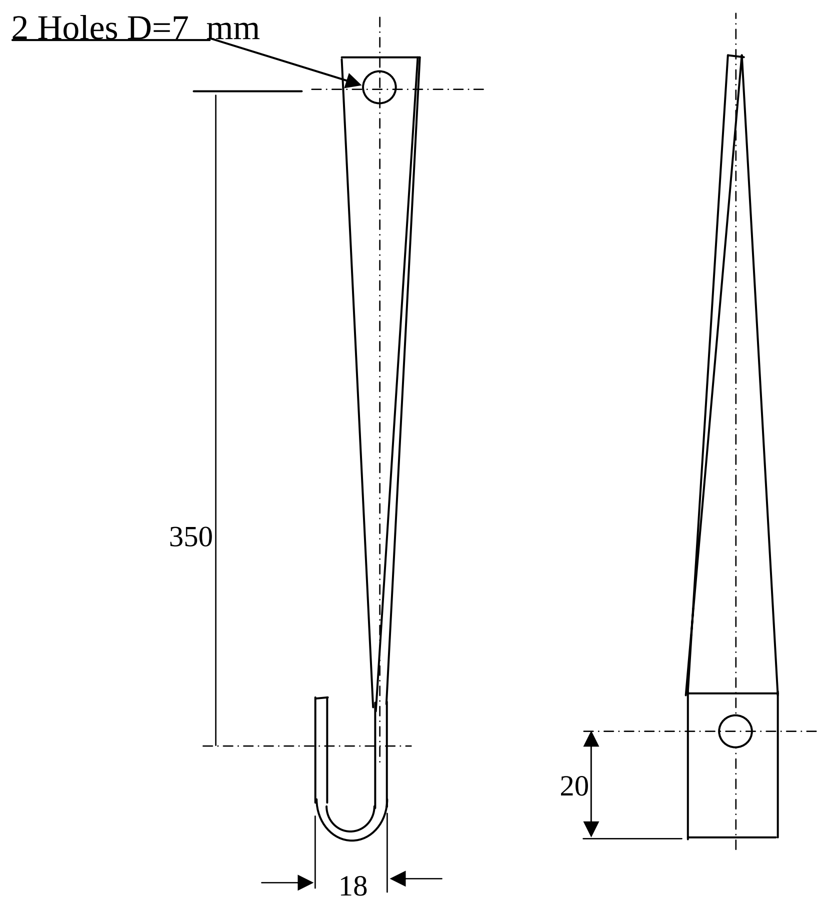
Material: Aluminum bar 25x3



Part no. 6

Quantity: 1

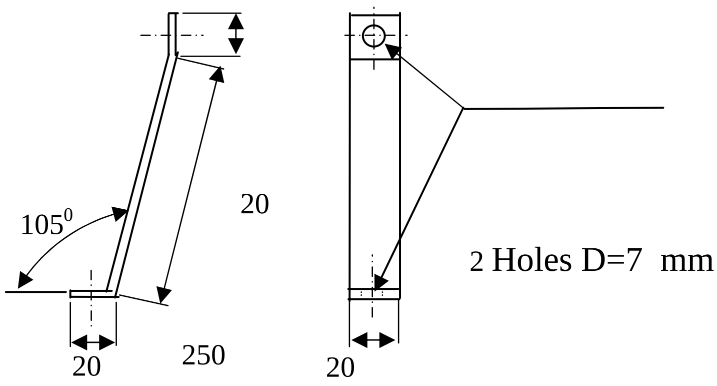
Material: Aluminum bar 25x3



Part no. 7

Quantity: 2

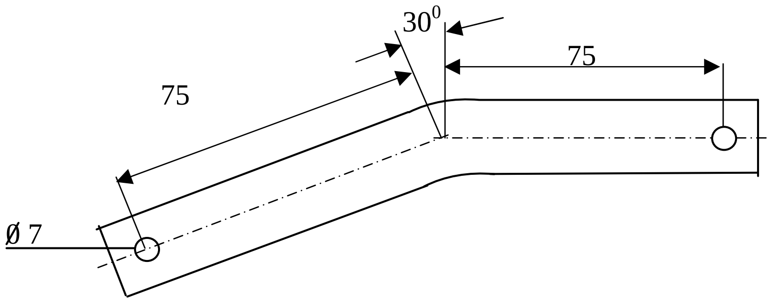
Material: Aluminum bar 25x3



Part No. 8

Quantity: 4

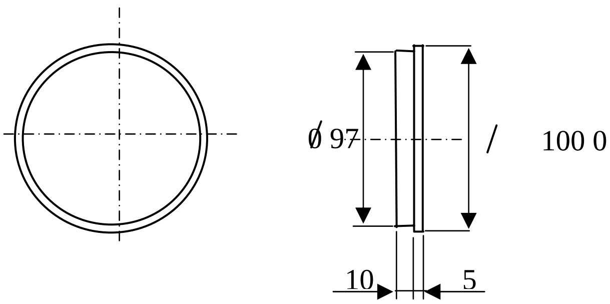
Material: Aluminum 25x3



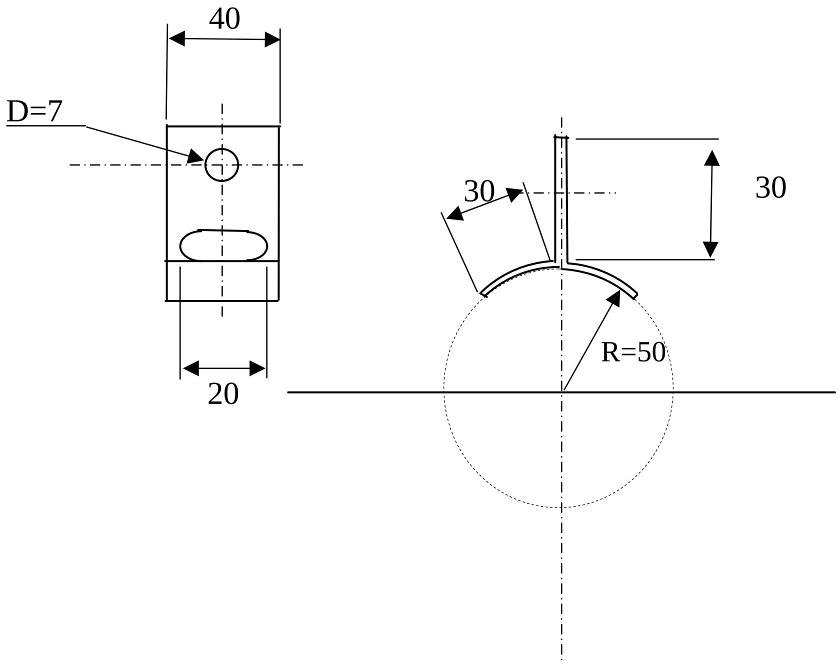
Part no. 11

Quantity: 1

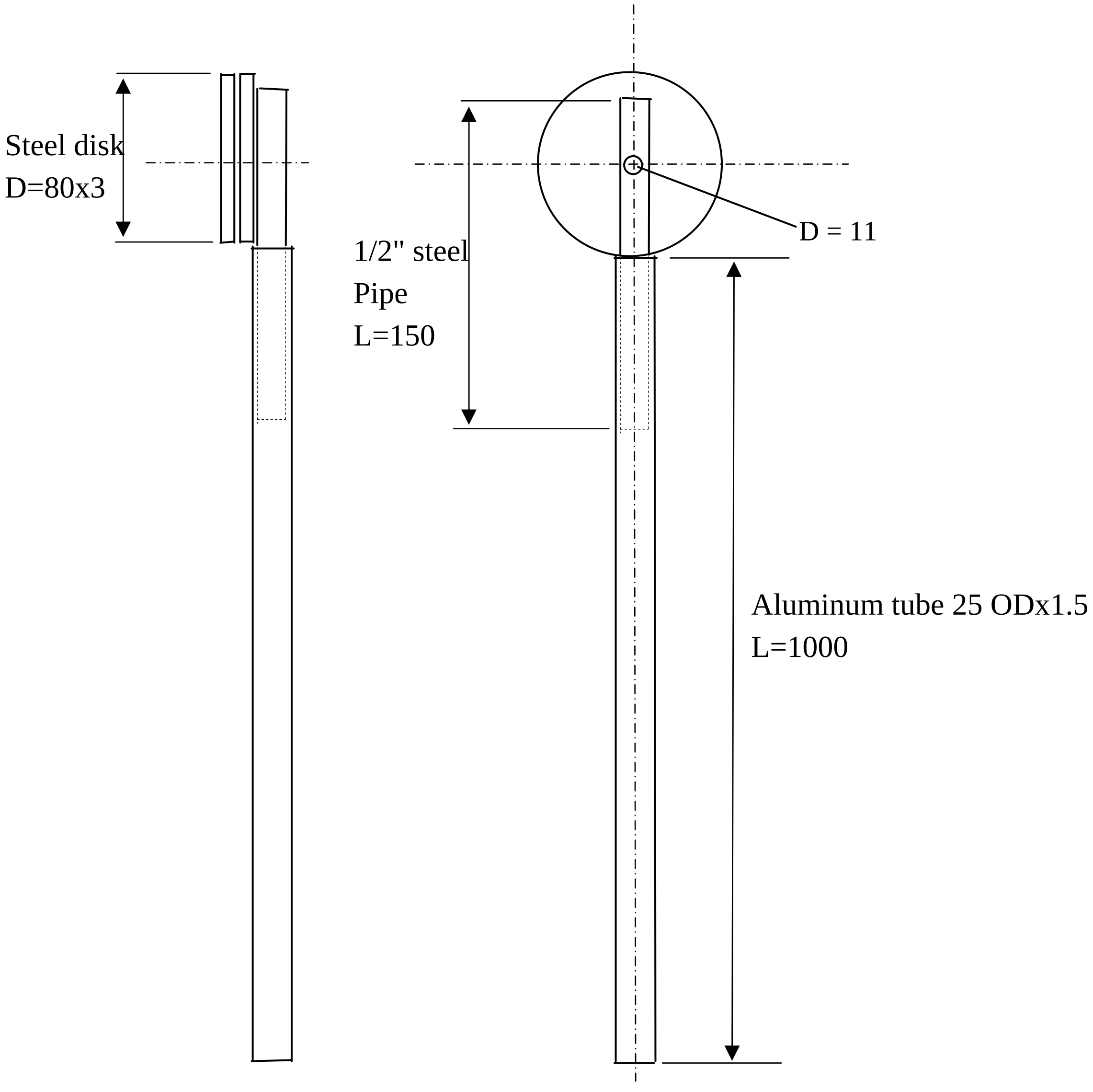
Material: Delrin



Part no. 15
Quantity: 6
Material: stainless steel 40x1.5



Part no. 16
Quantity: 2
Material: Aluminum tube + Steel plates and tube



Assembly and operation instructions

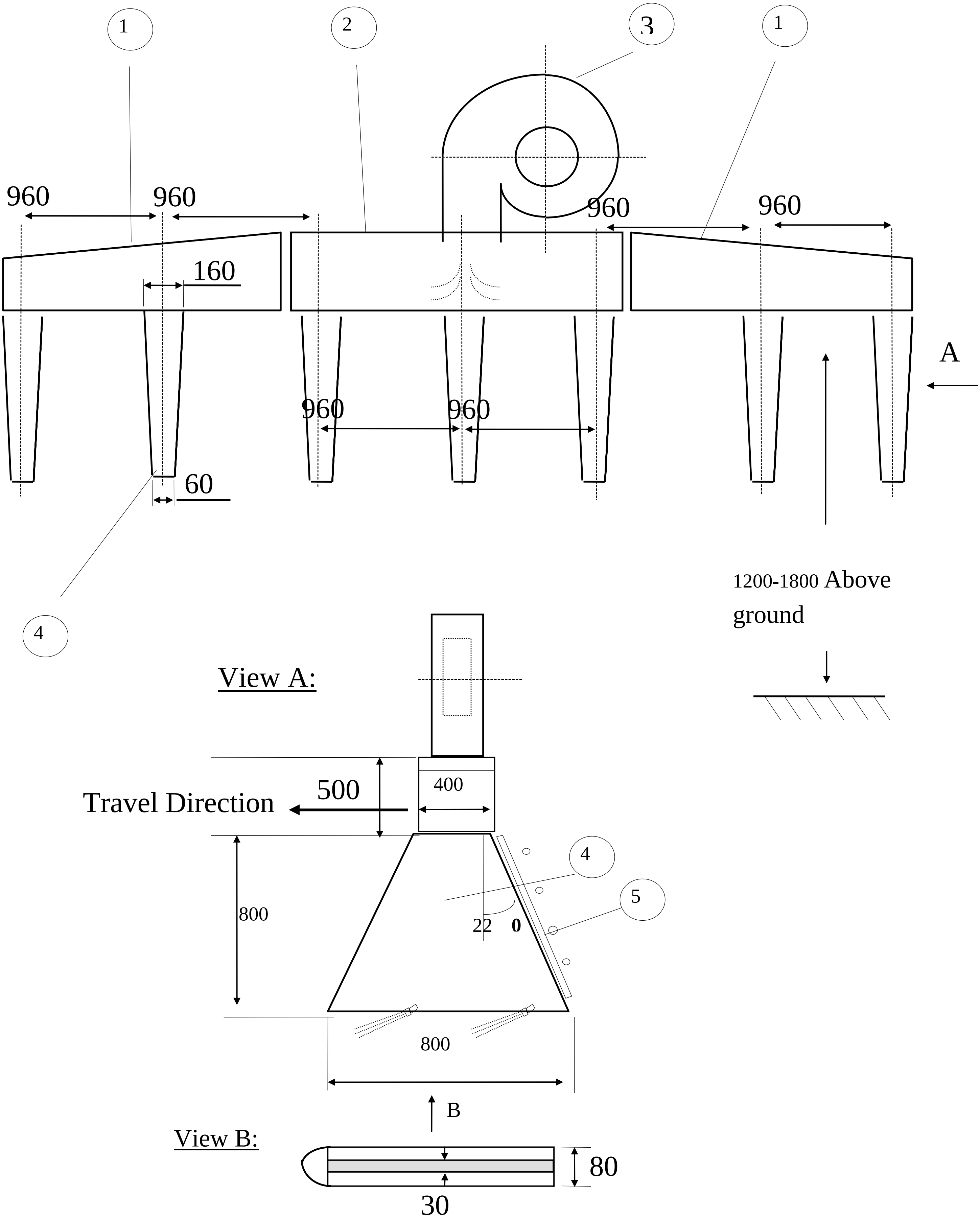
Assembly

1. The battery negative pole should be connected to the sprayer frame. One pole of all the Micron Ulva+ atomizers and the blower should be connected to the frame and all of them should be connected in parallel. A main switch should be connected on the positive pole of the battery.
2. The blower rotates in the direction, which provides the maximal airflow rate.
3. The flow rate of each nozzle should be adjusted between 200 to 260 cc per minute and the total flow rate of the sprayer should be between 900 to 1000 cc per minute.
4. The angles of the spinning disk should be set so that the disk axis is almost parallel to the ground. The end with the screw should be slightly lower than the end with the electric motor. The sprayer picture shows that the disk axis is tilted by approximately 10 degrees to the horizontal.

Operating instructions

1. The battery should be charged with a 12-Volt charger for 4 hours at low current of up to 3 Amps. With an automatic charger the battery can be charged over-night before each spraying day. Charging can be performed while the battery is in place and the main switch is turned off.
2. Place the sprayer on its legs.
3. Remove the protecting covers of the spinning atomizers before operating the sprayer.
4. Dilute the spray mix to get an active material of the recommended amount. Typically it is recommended to apply 16 liter of active material with 286 liter of water per hectare, resulting with 302 liter of spray liquid per hectare.
5. Fill the spray tank with the liquid to be sprayed.
6. Turn the water valve on and make sure that the liquid drains out from all spinning disks. Turn on the electric switch and check that all the spinning disks and the blower are turning.
7. The sprayer should be positioned on the operator's back.
8. The sprayer lower tip should have around 10 cm ground clearance. When the sprayer is strapped to the operator, adjust the tube connectors to get the desired ground clearance.
9. Open the liquid tap and the main switch and start walking. Operate the sprayer at a walking speed of 2 km/hr. The sprayer delivers approximately one liter per min. This gives 60 liters per hour or 300 liter per hectare. This amount of liquid is fairly high but was found to be the necessary minimum for efficient control with the developed oils and with moderately infested fields. Frequently check that the liquid leaves of the spinning disks as a mist.

3. Tornado Sprayer for Cotton Assembly and operation instructions



Parts List:

Part No.	Part Name
1	Lateral Air Duct
2	Central Air Duct
3	Blower
4	Drop-Tube
5	Spray Boom with X3 Nozzles

Technical data and assembly instructions

1. Air velocity at the outlets should be approximately 36 m/s.
2. The total area of the outlets is 1600 cm^2.
3. The blower capacity is 5.76 m^3/s.
4. The liquid mixing should keep operating at all times after the material was placed in the tank, to prevent material separation.

Operating instructions

1. Fill the spray tank with the spray liquid: Dilute the spray mix to get active material of the recommended amount. Typically, it is recommended to apply 16 liter of active material with 336 liter of water per hectare, resulting with 300 liter of spray liquid per hectare.
2. For optimal application, the tractor speed should be 4 km/hr. This ground speed should provide at least 350 liter/hectare.
3. The sprayer' slower end should have around 60 cm ground clearance.

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