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ARRIVÉE

Evaluation of a virus–insecticide combination for cotton pest control in Togo

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A low dose of cypermethrin combined with *Mamestra brassicae* virus was applied weekly against the bollworms *Helicoverpa armigera* (Hübner) and *Diparopsis watersi* (Rothschild) in smallholder cotton plots in northern Togo in 1989 and 1990. The timing of treatments was based on action thresholds. One or two additional sprays of *Bacillus thuringiensis* were applied to control *Syllepte derogata* F., or dimethoate to control aphids. No significant yield differences were observed between the plots treated with the pyrethroid–virus combination and the control plots, treated with the recommended insecticide schedule. However, the latter had a significantly higher percentage of healthy bolls. The virus applied was not reisolated from insects collected in the field.

Keywords: combined control; *M. brassicae* virus; insecticides; cotton; Togo; smallholder plots

The problems linked with the use of chemicals against crop pests are well known – resistance to the active ingredients applied, creation of imbalances within the fauna that lead to a resurgence of secondary pests, and environmental pollution and its toxicological consequences. With cotton, the case of resistance in the noctuid *Helicoverpa* (=*Heliothis*) *armigera* (Hübner) has been studied in particular detail (Kay, 1977; Gunning *et al.*, 1984; Collins, 1986; McCaffery *et al.*, 1989).

Current studies on cotton are searching for other control methods, including the use of biological agents such as insect viruses and bacteria. In the insect virus field, work by the Institut de Recherches du Coton et des Textiles exotiques (IRCT) began almost 30 years ago, mainly in Cameroon, Chad and the Côte d'Ivoire (Angelini and Vandamme, 1969; Atger, 1970; Atger and Chevalet, 1975; Jacquemard, 1982; Croizier *et al.*, 1983; Angelini and Jacquemard, 1984; Renou, 1987; Montaldo, 1991).

In the laboratory, Jacquemard (1978) has demonstrated the effect of the nuclear polyhedrosis baculovirus of the noctuid *Mamestra brassicae* L., isolated by Atger (1962), on the noctuid *Diparopsis watersi* (Rothschild). Third- and fourth-instar larval mortality from virus disease reached 100% at the end of the sixth day after being fed with 'green organs' soaked in a polyhedral suspension. Biache and Severini (1986) showed the effectiveness of the same virus on *H. armigera* second-instar larvae, fed on an artificial medium to which a polyhedral suspension had been added.

The *M. brassicae* virus, used alone, has been tested successfully against *H. armigera* on tomato in the field and glasshouse in Senegal (Biache and Severini, 1986) and in France (Biache and Chaufaux, 1986; Bues *et al.*, 1988; de Coninck and Biache, 1989). On cotton, experiments have generally been carried out at research stations, in small plots (80–100 m^2). The use of a virus alone did not give adequate control.

The combination of a 'low dose' of insecticide (i.e. at a lower dose than that recommended), applied weekly with a virus and known as 'combined control', was studied in Côte d'Ivoire as early as 1965 (Vandamme and Angelini, 1966).

In the laboratory, potentiation of the virus was studied by Ferron, Biache and Aspirot (1983), in particular with the *Spodoptera littoralis* (Boisduval) baculovirus and deltamethrin. These authors report that synergism was seen in *Heliothis virescens* (F.) between the action of *M. brassicae* virus and low doses of deltamethrin. In the field, a trial combining the use of the virus and deltamethrin was conducted at the Chad station in 1981, with positive results (Biache and Chaufaux, 1986).

The first large-scale smallholder trials were conducted in Cameroon in 1988 (J. P. Deguine, G. Ekukole and T. Montaldo, personal communication). The cypermethrin–*M. brassicae* combination with 5 g ha^{-1} and 2.5 × 10^{12} polyhedral inclusion bodies (p.i.b.) ha^{-1} was applied ten times, at weekly intervals, and led to a yield equal to that obtained with cypermethrin used alone at 36 g ha^{-1} (five treatments at 2-week intervals).

Our study in 1989 and 1990 aimed to show that an adapted combined control programme can be as effective as chemical control and can reduce the cost of protection. The choice of the pathogen, *M. brassicae* baculovirus, was based on previous results and the

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availability of commercial preparations. For economic reasons the number of treatments was selected to ensure that the protection programme would cost no more than the recommended chemical control programme. The trials were in the north of the country, where *H. armigera* and *D. watersi* are predominant.

Materials and methods

Plot selection and agronomic history

In view of the high heterogeneity of plots observed at Dapaong in 1989, the low pest pressure and low yield potential of the site, the trial was duplicated in 1990 at Bandjeli near Kabou (*Figure 1*). This latter trial was monitored daily by one of us (P. Le G.), assisted by the observer who had monitored the 1989 experiment.

The smallholder plots were chosen after emergence of the cotton, based on criteria such as homogeneity, slope, correct plant population density, planting date within the regional average range, simple geometric shape and area of ~5000 m^2. Certain plots were rejected if cropping recommendations were not implemented effectively.

Table 1 gives details of the number of plots chosen each year, the previous crop, soil tilling before planting, planting date (10-day period), fertilizer applications (data are difficult to obtain), number of hoeing rounds and earthing up. In 1989, fertilizer was applied from the 21st to the 42nd day after sowing (DAS).

In 1990, a complete NPKSB fertilizer was applied from 16 to 56 DAS. Urea was applied from 33 to 64 DAS. Each year, the intended rates were 150 kg ha^{-1} of NPKSB and 50 kg ha^{-1} of urea. Each plot was split into two equal sections (~2500 m^2). The exact area of each section was measured using a surveyor's chain and a compass. One section received the recommended programme, the other section the combined control programme.

Table 1. Details of plots used for the experiments carried out at Dapaong (1989) and Kabou (1990), evaluating a virus–insecticide combination for cotton pest control

Plot details	1989	1990
Total number	12	20
Former crop		
maize	–	8
cotton	4	4
sorghum–millet	8	3
fallow	–	3
groundnut	–	1
fallow after crop	–	1
Soil tilled		
by hoe	5	14
by plough	7	3
by tractor	–	3
Planting time (June)		
first 10 days	–	4
second 10 days	8	16
last 10 days	4	–
Fertilizer + urea		
applied together	12	9
applied separately	–	11
One hoeing round	–	18
Two hoeing rounds	12	2
Earthing up	6	17

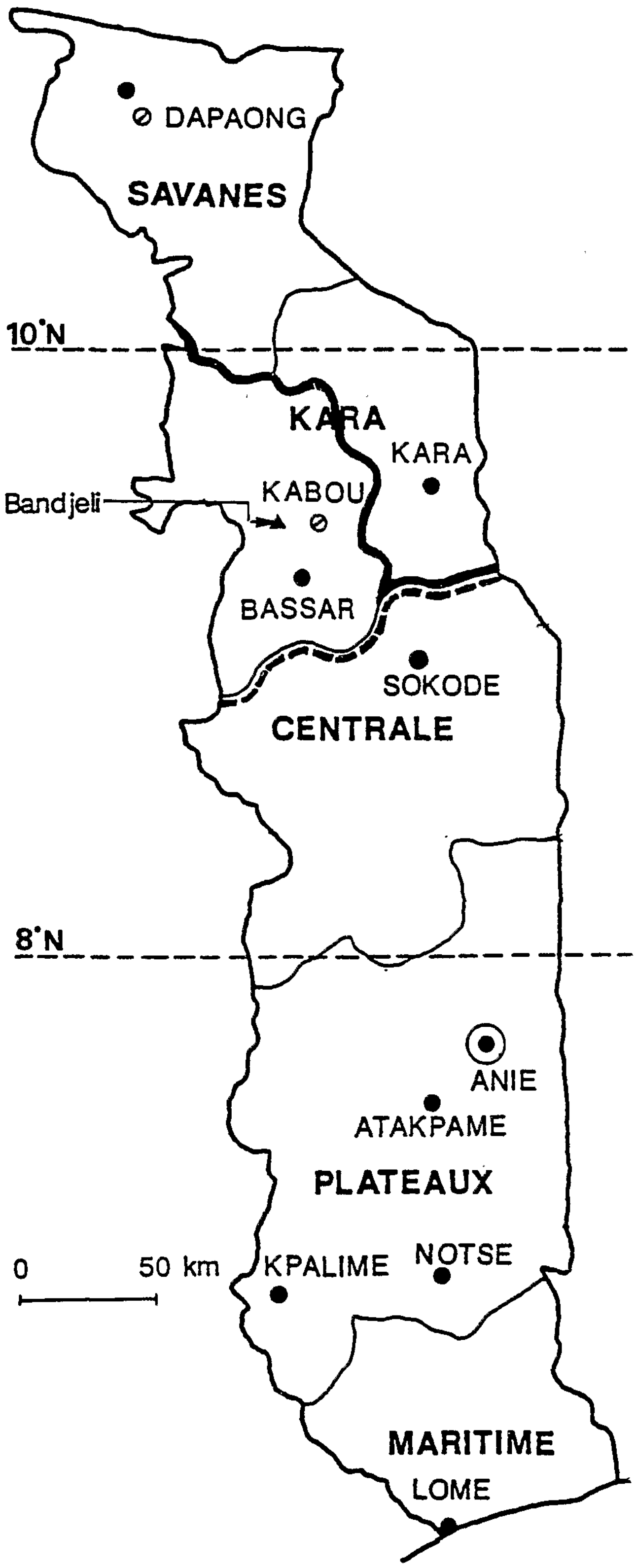

Figure 1. Experimental sites in the Republic of Togo in 1989 (Dapong) and 1990 (Bandjeli, near Kabou); ⦿, Research Centre; ——, edge of treatment zone, 1990; – – – –, edge of treatment zone, 1991; ————, regional borders

Protection programmes

Recommended programme (RP sections). In this programme, there were five 2-week treatments each year, the first being applied 50 DAS. The formulation contained 36–300 g l^{-1} of cypermethrin/dimethoate (ec formulations); 1 l ha^{-1} was applied using the Berthoud C8 hand-held battery-operated spinning-disc sprayer fitted with eight 1.5 V batteries and a green nozzle, diluted to an aqueous blend (LV 10 l ha^{-1}). Three rows were treated at a time.

Combined programme (CP sections). Treatments were based on action thresholds defined previously in Côte d'Ivoire and Zimbabwe (Sognigbe, 1990). The time of the first treatment to control the *H. armigera* and *D. watersi* was determined according to a threshold. The subsequent treatments (four or five) were mandatory. Treatments intended to control aphids and *S. derogata* were only applied once when the thresholds for each pest had been exceeded.

Crop monitoring began at the same time as the first treatment in the recommended programme. Twenty-five plants, chosen at random along the diagonal in CP sections, were observed and the number of plants with rolled leaves containing live *S. derogata* caterpillars was recorded, as well as the number of leaves containing aphids (four terminal leaves were observed on each plant) and the number of *H. armigera, D. watersi* and *Earias* sp. eggs and larvae.

Treatments were carried out if >30 leaves were infested with aphids, or if more than five plants were affected by *S. derogata*, or if more than three eggs or more than five larvae and eggs were observed.

The formulations applied were the following emulsions:

1. Mamestrin+, containing 3.6 g l^{-1} cypermethrin and 2.1×10^{12} p.i.b. l^{-1} *M. brassicae* virus, a UV radiation screen, an adhesive agent and a phagostimulant; 1 l ha^{-1} was applied against *H. armigera* and *D. watersi*;
2. Dimethoate, containing 300 g l^{-1} dimethoate; 1 l ha^{-1} was applied against *Aphis gossypii* Glover;
3. FORAY 48 B, containing 12.7×10^9 IU l^{-1} (11 300 IU mg^{-1}) *Bacillus thuringiensis* serotype 3a3b [biological quantitative analysis carried out on *Trichoplusia ni* (Hübner)]; 1.5 l ha^{-1} were applied against *Syllepte derogata* F.;
4. Isoxathion, containing 300 g l^{-1} isoxathion; 1 l ha^{-1} was applied against *S. derogata*. This formulation was only used in 1990 to treat half the CP sections (i.e. ten sections), the others being treated with FORAY 48 B.

These formulations were applied in the same way as that applied in the RP sections (LV 10 l ha^{-1}), with the same type of apparatus (Berthoud C8), using a unit reserved exclusively for the CP sections, never containing the formulations intended for the RP sections. Products were mixed in the field and the treatment apparatus tank was filled in two stages.

Given the economic criteria applied, the numbers of treatments allowed were as follows. In 1989, two treatments of dimethoate, if necessary, one treatment of *B. thuringiensis*, if necessary, and five treatments of Mamestrin+ at weekly intervals, were applied. All the sections were treated if a threshold was exceeded in >50% of them, except for the single, targeted treatment against *S. derogata*. In 1990, the numbers of treatments were three of dimethoate, if necessary, two of *B. thuringiensis*, if necessary, two of isoxathion, if necessary and five (at weekly intervals) of Mamestrin+, with a sixth treatment applied if necessary. Each section was monitored and treated individually.

The Kabou region is also affected by *Cryptophlebia leucotreta* (Meyrick) and *Pectinophora gossypiella* (Saunders), the larvae of which develop inside the bolls. Treatment against the pests was applied 2 weeks after the last treatment in the RP sections, using cypermethrin at a rate of 36 g a.i. ha^{-1}.

Data collected

The volumes of spray actually applied during each treatment, and number of plants ha^{-1} were recorded.

In 1990, in each section, 100 green bolls were taken at random along a central row 110 DAS to assess the amount of damage. Green bolls with a diameter > 20 mm were harvested from every fourth plant. A distinction was made between bolls with holes bored by exocarpic larvae (*H. armigera*, *D. watersi*, *Earias* sp.) and those pierced by endocarpic *C. leucotreta* or *P. gossypiella*. In addition, pricks made by heteropterans can be seen inside the carpels and thus enabled pricked bolls to be distinguished.

Analysis of ripe bolls was carried out after total opening. The bolls were chosen in four 10 m segments of cotton plants, ten rows apart, chosen along the diagonal in each section. A little over 500 ripe bolls per section were collected in this way and analysed. Two additional boll categories (rotten bolls and mummified bolls) were defined.

In 1989, larvae of *H. armigera* (155), *D. watersi* (50), *S. littoralis* (31) and *Earias* sp. (nine), found in the CP sections were reared in an attempt to find the virus applied, or to detect an indigenous virus. In 1990, only four *H. armigera* larvae, five *D. watersi* larvae and one *Earias* sp. larva were found in the field and reared.

Lastly, seed cotton yields for the RP and CP sections were calculated after weighing the total harvest for the areas measured, using a spring balance. An analysis of variance was carried out each year with seed cotton yield variables and in 1990 when the variables were percentage of bolls in each category (Bliss transformation) and number of plants ha^{-1}. In 1990, for yield, the analysis of variance was initially carried out with 19 data pairings, and subsequently taking the sections due to be treated against *S. derogata* using isoxathion (10 pairings) or *B. thuringiensis* (nine pairings).

Results

Jacquemard (1982) has re-isolated the virus in the target pest populations after application. In our trial, rearing *H. armigera*, *D. watersi*, *S. littoralis* and *Earias* sp. larvae collected in the field did not reveal any viruses in either year. The true level of virus infection in the field is unknown.

Treatment operations

The average amount of active ingredient applied per hectare and per treatment (*Table 2*) were similar to the recommended doses. In 1990, the volumes applied were slightly higher than recommended, as the farmers who took part in the study did not have to pay the 'insecticide' subscription that year.

Protection programme in the CP sections

In 1989, the aphid threshold was exceeded at the start of the programme, and two dimethoate treatments were applied, 50 and 75 DAS (i.e. on the dates of the first two treatments in the RP sections). The *S. derogata* threshold was exceeded 75 DAS in two plots, but in the other plots, the treatment was applied at the same time as the third treatment in the RP sections. The Mamestrin+ treatment programme began 75 DAS. The crop was therefore no longer protected at the end of the cycle, as the fifth and last treatment took place at the time of the fourth treatment in the RP sections.

In 1990, the *H. armigera* and *D. watersi* caterpillar threshold was never reached at Kabou. The five Mamestrin+ treatments were applied to protect the crops at the end of the cycle, from a week after the third treatment in the RP sections.

The timing of treatments against aphids and *S. derogata* in 1990 (*Figure 2*) shows that aphid infestation varied considerably. The three planned treatments were carried out, except in two CP sections which were given only two treatments. In 17 out of 20 CP sections the threshold was exceeded for a fourth time, but by 90 DAS population levels had fallen in the plots as a whole.

In contrast to treatments against *A. gossypii* at the start of the season, *S. derogata* thresholds were exceeded at the end of the growth cycle. Of the 19 treatments carried out, 16 were applied after the fourth treatment in the RP sections. Seven (of 20) sections were not protected against *S. derogata*. The sections treated against this pest were given two applications. In only one section would an additional treatment have been necessary as the threshold had been exceeded.

Boll analysis (*Table 3*)

The RP sections contained a much higher percentage of healthy bolls (green or ripe) than did the CP sections. The percentage of bolls (green or ripe) damaged by *C. leucotreta* or *P. gossypiella* larvae was higher in the CP sections. The percentage of ripe bolls with bollworm

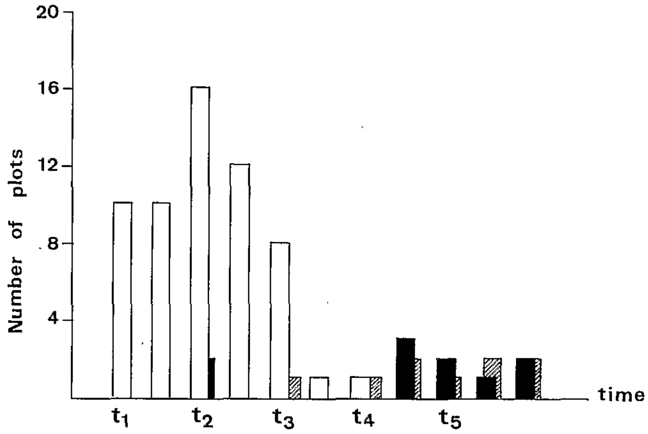

Figure 2. Numbers of plots treated in 1990 against *A. gossypii* (□) or *S. derogata* with *B. thuringiensis* (■) or isoxathion (▨); t_1–t_5 represent dates of treatments carried out every 2 weeks in the recommended programme (RP) sections

Table 2. Average amount of active ingredient (a.i.) of (A) recommended cotton pest control treatment and (B) a combined control programme, actually applied per hectare and per treatment in 1989 and 1990 in Togo

Year	Programme	Treatment	Amount applied (g a.i. ha^{-1}) Mean	Range
1989	A	Cypermethrin	36.8	33.1–43.6
		Dimethoate	307.0	276.0–363.0
	B	Cypermethrin	4.2	3.9–5.0
		Dimethoate	311.7	288.0–381.0
		B.t.[a]	19939 (IUAK[b] ha^{-1})	18415–23749
		M. brassicae virus	2.1×10^{12} (CIP ha^{-1})[c]	1.9×10^{12}–2.5×10^{12}
1990	A	Cypermethrin	36.5	34.2–39.7
		Dimethoate	303.8	284.9–331.2
	B	Cypermethrin	4.2	3.4–4.8
		Dimethoate	317.7	258.8–389.7
		B.t.	19592 (IUAK ha^{-1})	18317–22108
		M. brassicae virus	2.1×10^{12} (CIP ha^{-1})	1.9×10^{12}–2.4×10^{12}
		Isoxathion	257.5	215.3–284.3

[a]B.t., *Bacillus thuringiensis*; [b]IUAK, International Units of *Anagasta kühniella*; [c]CIP, corps d'inclusion polyédriques (= polyhedral inclusion bodies)

Table 3. Results of green and ripe boll analyses carried out at Kabou in 1990 after application of a recommended programme (RP sections) or combined programme (CP sections) of treatment for cotton pest control

	Green bolls				Ripe bolls							Total no. of larvae[c]	
Sections	No. analysed	Healthy (%)	Pierced by bollworms (%)	Pricked by heteropterans (%)	No. analysed	Healthy (%)	Pierced by exocarpic larvae[a] (%)	Pierced by endocarpic larvae[a] (%)	Pricked by heteropterans (%)	Rotten (%)	Mummified	C	P
RP	2000	71.0	3.4	26.0	13913	48.2	2.6	22.5	13.0	11.0	2.8	11	14
CP	2000	58.7	8.3	33.7	13526	38.7	2.6	28.7	15.4	12.0	2.7	49	54
F		10.2**	12.7**	5.9*		11.9**	0.03	8.4**	3.8(10%)	0.6	2.1	Not analysed	
CV[d](%)		13.9	41.8	20.3		13.5	30.0	16.4	16.8	17.3	23.0		

[a]*H. armigera, D. watersi, Earias* sp.; [b]*C. leucotreta, P. gossypiella*; [c]C, *C. leucotreta*; P, *P. gossypiella*; [d]CV, coefficient of variation. *,** differences significant at the 5% and 1% level, respectively (or, where indicated, at the 10% level)

damage was ten times lower than the percentage of pierced bolls, which reflects the low infestation levels of the pests targeted by the study in 1990. There was high pest pressure on the bolls, as even on the RP sections < 50% of ripe bolls were healthy.

Number of plants ha^{-1} and seed cotton yields

An analysis of the variable 'number of plants ha^{-1}' in 1990 did not show any statistically significant differences between the RP and CP sections ($F = 2.28$). The mean yields obtained in 1989 in the RP and CP sections at Dapaong were 740.0 and 651.8 kg ha^{-1}. There was no significant difference in production ($F = 2.30$), but the coefficient of variation was high (20.5%).

In 1990, at Kabou, the difference in yields (*Table 4*) between the RP and CP sections was significant ($F = 3.59$) only at the 10% level. If the sections treated with isoxathion or *B. thuringiensis* are considered separately, there is a difference for the latter only, at the 10% level.

Discussion

These trials were in two distinct ecological areas that affected pest incidence. At Kabou in 1990 the very low incidence of bollworms (*H. armigera* and *D. watersi*) contrasted with large numbers of *C. leucotreta*, which were not found at Dapaong in 1989.

At Dapaong, three treatments (rather than the five recommended) were possible without significant production losses, as pest pressures were moderate in the year when the experiment was carried out.

Other trials examining the possibility of using less pesticide in 'dose-frequency' protection programmes (Aspirot and Menozzi, 1985; Deguine and Silvie, 1988) have shown that two-thirds of the recommended amount of active ingredient per hectare, applied weekly, rather than once every 2 weeks, has given satisfactory results. According to Aspirot and Menozzi (1985), more frequent applications make it possible to treat the first larval instar, when the parts are more susceptible to the low insecticide doses. This partly explains the effectiveness of the combined control programme, in which applications are made weekly, once the programme has started, but with one-tenth of the recommended cypermethrin dose.

At Kabou, the high pressure from *C. leucotreta* and *P. gossypiella* in the CP sections is also worth noting. The combined programme, when assessed in terms of amount of damage and number of larvae, was not as

Table 4. Seed cotton yields per plot (kg ha^{-1}) at Kabou (1990) and results of analysis of variance after application of a recommended programme (RP sections) or combined programme (CP Sections) of treatment for cotton pest control
(a) Analysis 1

Plot no.	RP sections	CP sections
1	1486	1319
2	(772)[a]	(1394)[a]
3	1294	1275
4	1089	1148
5	1229	990
6	837	843
7	1361	1093
8	1292	1416
9	1108	875
10	1228	930
11	873	920
12	1255	1359
13	899	939
14	903	822
15	928	881
16	1058	1039
17	959	1037
18	1084	1029
19	1073	1143
20	1219	968
Mean	1114.6	1054.0

$F = 3.59$ (10%)
CV = 9.09%

[a]Plots omitted from the analyses of variance for agronomic reasons

(b) Analysis 2: combined data for treated sections

Treatment	RP sections	CP sections
Isoxathion	1042.0	1024.0
$F = 0.24$ CV = 7.5%		
B. thuringiensis	1180.0	1081.0
$F = 3.96$ (10%) CV = 9.8%		

effective as the recommended programme, but the yields obtained did not differ significantly. The late triggering of the combined programme (after the third treatment in the recommended programme) and the possibility of cotton compensating for the losses incurred at the start of the cycle at the end of the cropping period, may explain these results. However, implementing this programme means greater survival of non-target fauna, particularly ladybirds. A saving of more than two-thirds of pyrethroid is made, compared with the amount of pyrethroid applied in the control programme, taking into account the additional cypermethrin treatment at the end of the cycle.

The number of plots observed at Kabou was only 19, whereas to show a difference in production of 100 kg ha^{-1} between the two programmes in 80 cases out of 100, it would have been necessary to have 32 plots (calculations carried out using STATITCF software). A study on such a large scale calls for considerable human resources and would need to be undertaken in the savannah region, representing 12 000 hectares, i.e. 15% of the 80,000 hectares planted with cotton in Togo [source: SOTOCO (Société Togolais du Coton)].

Implementation of the observation method adopted in this study will pose several problems for growers. Besides the problem of pest or damage identification, ~40 min is needed to examine 25 plants. If this type of programme were to be extended on a large scale, grower training would be essential. Furthermore, growers should pay only for the amount of products actually applied, rather than for the full recommended programme as at present.

Acknowledgements

The authors are grateful to the Calliope company (17 rue du Chapeau Rouge, 34500 Béziers, France) for the facilities made available for this study, and to Mr Mivessonou for the quality and diligence of the work carried out.

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Received 15 May 1992
Revised 3 February 1993
Accepted 5 February 1993