MANAGEMENT OF BLACK SIGATOKA IN CAMEROON

L. de Lapeyre de Bellaire[1, 2], J. Essoh Ngando[2], C. Abadie[3] J. Carlier[4] and E. Fouré[1, 2]

Summary

Introduction. In Cameroon, *Mycosphaerella fijiensis* was reported for the first time in 1981 and the control against black leaf streak disease represents the highest production cost which can attend up to 10% of total production cost. Towards the end of the 80s, a forecasting system using biological descriptors was elaborated and applied with success, reducing the number of treatments to 12 – 14 per year. Since 1996, the development of fungicide resistance lead to the progressive abandon of this rational strategy at the expense of systematic control methods. Thus in 2005, about 40-50 treatments were done on most of the plantations and the control program was based on the ratio of 90% of contact fungicides and 10% of systemic and penetrant fungicides. This evolution has lead to an important increase of the cost of disease control, but also to an increase of negative environmental effects.

Material and Methods. In those conditions, research conducted in Cameroon is aimed to: (1) Experiment fungicides having less negative environmental effects; (2) Adapt control strategies to the situation of fungicide resistance; (3) Evaluate the adaptive potential of *Mycosphaerella fijiensis* populations in response to the use of systemic fungicides.

Results. (1) Some of the new fungicides tested enabled an acceptable control of BLSD when they were used in oil. (2) Experiments realised in 2005 have shown that chlorothalonil applied on a systematic framework, and compared with penetrants used in a forecasting strategy, enabled a very good control of the disease, even in the rainy season: strong reduction of the Stage of Evolution of the disease, increase of phytosanitary level in the banana plantations (Youngest leaf spotted). (3) Very important fluctuations in the levels of resistance to systemic fungicide have been observed in Cameroon. Some observations clearly show that management of resistance should refer to the control of the disease in nurseries, and also to nurseries location.

Discussion. New fungicides should be used in water alone to enable a rapid conversion to chlorotalonil applications and to reduce negative effects of oil when used in a systematic calendar (treatments every week). New strategies where the proportion of chlorothalonil would be rationalized to a minimum will be evaluated in the next year. We make the hypothesis that the fluctuations observed in the resistance frequency to systemic fungicides could result from a lower fitness of resistant strains, which could be counter- selected in the absence of the fungicide selection pressure and/or gene flow between the treated and the untreated areas.

Corresponding author : L. de Lapeyre de Bellaire (luc.de_lapeyre@cirad.fr)

Introduction.

Black leaf streak disease (BLSD) is one of the main parasitic constraints in agro–industrial plantations of dessert bananas. This disease has an important impact, since all banana varieties cultivated in agro–industrial plantations belong to the Cavendish sub group which is highly susceptible to BLSD (Fouré *et al*., 1990). On susceptible varieties of bananas and plantains, BLSD provoke the appearance of leaf necrosis, and the attack of the pathogen (*Mycosphaerella fijiensis* Morelet) can lead to important reduction in photosynthetic activity and yield losses which vary between 10 and 100%. However, the most important effect of the disease is indirect, for bunches harvested on highly affected plants have their green life reduced and cannot be exported (Stover, 1974). This foliar disease is present in the major banana producing countries in the world (Pasberg-Gauhl *et al*., 2000), and the intensive cultivation of dessert banana for exportation can only be achieved through intensive chemical control.

[1] CIRAD, Unité de Recherche 'Systèmes de culture bananes, plantains et ananas'
[2] CARBAP, Centre Africain de Recherches sur les Bananiers et Plantains, Centre de recherches de Njombé, Bureau de liaison BP 832, Douala, Cameroun.
[3] CIRAD, Unité de Recherche 'Amélioration génétique d'espèces à multiplication végétative', Station de Neufchâteau, 97130, Capesterre belle eau, Guadeloupe.
[4] CIRAD, UMR-BGPI, Campus International de Baillarguet, 34398, Montpellier, Cedex 5

Control of BLSD is essentially based on aerial application of fungicides to protect the young leaves from contamination. In most production countries, fungicides are applied systematically following a predetermined framework (Marin *et al.*, 2003). In some cases, forecasting systems have been established and treatments are decided as a function of the degree of evolution of the disease (Fouré, 1988; Bureau, 1990). Two types of fungicides are used: (i) contact fungicides which have only preventive effect, (ii) penetrating or systemic fungicides which have more or less curative effect. The latter are antimitotics (benzimidazoles), inhibitors of ergosterol biosynthesis of group 1 (triazoles) and of group 2 (morpholines) and respiratory inhibitors (Qo inhibitors or strobilurines). Moreover, these fungicides particularly the systemic ones are used in oil (or in an oily emulsion) for these oils enable a better distribution of the fungicide, limiting it from being wash away by rainwater, and particularly because they have a fungistatic effect. Finally, in the treatment strategies, fungicides are used in alternation or in mixtures (Marin *et al.*, 2003). Depending on prevailing climatic conditions which determine parasitic pressure, the susceptibility of the parasite to systemic fungicides and the control strategy used, 12 to 60 treatments per year are realized for BLSD control.

In Cameroon, *Mycosphaerella fijiensis* was reported for the first time in 1981 (Fouré, 1984) and the control against black leaf streak disease represents the highest production cost which can attend up to 10% of total production cost. In fact the epidemiological context which exists in Cameroon, particularly in the Mungo division where most of the banana plantations are located, is very favourable to the disease notably in the rainy season from May to December (Fouré and Moreau, 1992). Towards the end of the 80s, a forecasting system using biological descriptors was established (Fouré, 1988) and enabled a good control of the disease with only 12-14 applications per year using systemic fungicides (figure 1). Since 1996, the breakout of fungicide resistance lead to the progressive abandon of this rational strategy at the expense of systematic control methods. Thus in 2005, about 40-50 treatments were done on most of the plantations and the control program was based on the ratio of 90% of contact fungicides and 10% of systemic and penetrant fungicides (figure 2). This evolution has lead to an important increase of the cost of disease control, but also to an increase of negative environmental effects. The objective of this paper is to present some solutions to the constraint of fungicide resistance, for BLSD control. This refers to (1) experiment fungicides having less negative environmental effects; (2) adapt control strategies to the situation of fungicide resistance; (3) evaluate the possible reversibility of fungicide resistance in order to reintroduce a forecasting control strategy using systemic fungicides.

Material and Methods

1. *Evaluation of bio-fungicides*

Various bio-fungicides have been experimented in order to evaluate their effect on BLSD and to identify on what kind of strategy they could be used for the control of this disease. Fungicides having a strong curative effect (systemic fungicides) can be used in a forecasting strategy, since those having a low curative effect (contact fungicides) require a systematic strategy. These tests have been carried out in the course of 2005, on the experimental station of CARBAP in Njombe, on banana plots grown with the cultivar Grande Naine, triploïd AAA of sub-group Cavendish, which is highly susceptible to BLSD. Natamycin, Timorex (essential oil of *Melaleuca alternifolia*), and Serenade (*Bacillus subtilis*) were compared in separate experiments with reference fungicides and/or an untreated control (table 1).

For each experiment, the treatments were repeated 3 times on plots of 60 to 100 banana plants, and 10 plants located in the centre of the plots were selected and observed for BLSD evaluation. The Stage of Evolution of the Disease (SED) and the Youngest Leaf Spotted (YLS) were scored every week:

- The SED was evaluated as in the method described by Fouré (1988). This parameter is an indicator of the dynamics of the disease and is helpful to observe the immediate effect of fungicide applications. In this method, the young attacks of the disease are quantified by scoring the most advanced stage of the disease according to Fouré's scale (1982) on the leaves n°2 to 4. A coefficient attributed to all (leaf number) / (stage of the disease) association, enables the calculation of the SED (table 2).

- The YLS has been scored according to Stover's method (Stover and Dickinson, 1970). This parameter is helpful to express the efficiency of the fungicide control through the development of necrotic lesions.

Fungicide applications were realized on a 7-10 days framework with a hand sack atomizer, using a volume of 20L of either oil, water or an emulsion (7L oil + water) per hectare.

For each experiment, the mean values of SED and YLS of the different treatments were calculated for the different weeks of observation. These values were submitted to an ANOVA, and to a test of Tukey at the level of 5%.

2. Adaptation of control strategies

The objective of these trials was to evaluate new strategies excluding the use of systemic fungicides for BLSD control in the commercial banana plantations of Cameroon. Those strategies would then rely on contact (mancozeb, chlorothalonil) or penetrant (pyrimidins, sterol biosynthesis inhibitors of group 2) fungicides. The experiment was carried out on a commercial banana plantation of 200 ha divided in two equal parts for aerial spraying, in order to compare two different strategies in the dry season and in the rainy season (figure 3).

2.1. Dry season strategies

The objective was here to investigate the possibility to control BLSD in the dry season using a forecasting strategy with penetrant fungicides. Two strategies were then compared from January to April 2005:

- a strategy where fungicide applications were decided according to the level of the SED (Foure, 1988). The fungicides used in this strategy were either sterol biosynthesis inhibitors of group 2 (Calixin or Impulse), either Pyrimidins (Siganex). Calixin and Impulse were applied in straight oil (15 l/ha), and Siganex was used in an emulsion of 7L oil + 8L water + emulsifier.
- a strategy where chlorothalonil (Bravo 720) was used systematically on a 7 day timetable. Bravo was applied in 20L of water/ha.

2.2. Rainy season strategies

The objective was here to evaluate the possibility to control BLSD in the rainy season using penetrant and contact fungicides in a systematic strategy. Two strategies were compared from June to December 2005:

- a strategy where penetrants were used systematically on a 10 days timetable. The fungicides used in this strategy were either sterol biosynthesis inhibitors of group 2 (Calixin or Impulse), or either Pyrimidins (Siganex). Calixin and Impulse were applied in straight oil (15 l/ha), and Siganex was used in an emulsion of 7L oil + 8L water + emulsifier.
- a strategy where chlorothalonil (Bravo 720) was used systematically on a 7 day timetable. Bravo was applied in 20L of water/ha.

2.3. Evaluation of BLSD

On each part of the trial, two plots of 10 plants were selected for weekly observation of the SED and of the YLS, as seen previously. Nevertheless, in commercial plantations, leaves spotted are often removed, and it is not possible to observe the YLS. Then, where no spotted leaves were observed, NL, the total number of leaves of the plant was noted, and the value NL+1 was considered as the YLS value for this plant. In fact, it is then considered that the older leaf has been removed in the course of sanitation programs and that this leaf was the YLS.

3. Status of fungicide resistance in commercial banana plantations of Cameroon

3.1. Methodology

Regular monitoring of fungicide resistance in the commercial banana plantations has been conducted since 1985 in order to detect any shift in the sensitivity to systemic fungicides in *M. fijiensis* populations. The methodology used was a germination test of ascospores projected on an agar medium amended or not with a specific fungicide concentration.

Leaf samples were collected in commercial banana plantations as well as in untreated plantations located in the same area. Each sample was constituted by leaf portions bearing lesions of stage 6 (Fouré, 1982) that were collected on 20-25 plants of the same plot. In the different years, leaf samples were collected in the same sectors of the plantations in order to observe the evolution of fungicide sensitivity in a same place over a long period.

At the laboratory, leaf samples were incubated in a plastic bag for 48h. Leaf pieces of 2cm x 2cm were then cut and bulked. 4-6 leaf pieces were stapled on a disc filter paper and immersed in sterile distilled water for 10 min. Paper discs were then placed in the cover of a Petri dish below the agar media amended or not with the fungicide, and 7 plates were used for a same fungicide concentration. After ascospores discharge (3 h), the paper discs were removed and the plates were incubated for 48 h and observed under a light microscope for ascospore germination.

3.2. Evaluation of resistance to antimitotics

These fungicides obstruct the functioning of the achromatic spindle indispensable for cell division. Sensitivity to benomyl or methyl-thiophanate was evaluated at the concentration of 5 ppm, and 100 ascospores were observed. Those having a distorted germ tubes or that did not germinate were considered as susceptible and those with normal germ tubes were considered as resistant. The proportion of resistant strains was then calculated. Data were observed on a same commercial banana plantation located in Njombe, from 1993 to 2004.

3.3. Evaluation of resistance to Sterol biosynthesis inhibitors of group 1

These fungicides inhibit the biosynthesis of ergosterol, a major component of fungi cell membranes, and inhibit the action of a C-14 di-methyl esterase. The action of these fungicides on germination is translated by a more or less important inhibition of the germination tube. Sensitivity to propiconazole was evaluated at the concentration of 0.1 ppm, and the germ tube length of 50 ascospores was measured with a micrometer on control (Lc) and fungicide amended medium (Lf). The growth inhibition (G.I.) was calculated as: G.I. = $[1-(Lf/Lc)]\times 100$, and the proportion of ascospores in different inhibition classes was also determined. Particularly, the percentage of ascospores falling in the classes represented in the untreated area population (high inhibition classes: GI > 50%) was calculated. Data were observed in a same commercial banana plantation located in Njombe from 1996 to 2005.

3.4. Evaluation of resistance to strobilurins

These fungicides inhibit respiration because of their affinity with cytochrome b. The resistance is conferred by mutation of a base of the mitochondrial gene coding for cytochrome b. This mutation is responsible for the change of an amino acid (glycine ---> analine) at position 143, resulting in the lost of affinity between the active ingredient and the cytochrome b (Sierotzki *et al*., 2000). Sensitivity to azoxystrobin was evaluated at the concentration of 1 ppm, and the germ tube length of 50 ascospores was measured with a micrometer on control (Lc) and fungicide amended medium (Lf). The growth inhibition (G.I.) was calculated as: G.I. = $[1-(Lf/Lc)]\times 100$, and the proportion of ascospores in different inhibition classes was also determined. Particularly, the percentage of ascospores falling in the classes represented in the untreated area population (high inhibition classes: GI > 70%) was calculated, because it has been demonstrated that such strains should be considered as susceptible strains to azoxystrobin (Amil *et al*., 2002). Azoxystrobin was introduced in spraying programs in 2001, and data were observed on a same commercial banana plantation located in Mbanga from 2001 to 2005.

Results

1. Evaluation of bio-fungicides

Timorex, essential oil of *Melaleuca alternifolia,* applied in water did not allow a good control of BLSD (table 3). Timorex applied in oil, controlled the disease as well as Siganex, but it is probable that this effect was due to the fungistatic effect of oil. Moreover, Timorex used at this rate was phytotoxic: brown spots of 1-3 mm were observed on leaves, particularly when Timorex was used in oil.

Natamycin in oil controlled the disease as well as Siganex, and this effect was highly significant from the untreated control as shown by mean values of SED and YLS (table 4). Nevertheless it is not possible to determine whether the efficacy of the Natamycin/oil treatment was due to the own effect of Natamycin and/or to the fungistatic effect of oil.

All treatments with Serenade (*Bacillus subtilis*) enabled a significant control of BLSD as compared with the untreated control (table 5). Serenade mixed with Dithane F488 had a significant better effect on SED than Serenade alone, but no significant differences were observed on the value of the YLS. Serenade treatments controlled the disease as well as straight oil and mancozeb mixed in oil. Nevertheless it is not possible to determine whether the efficacy of the Serenade treatments was due to the own effect of *Bacillus subtilis* and/or to the fungistatic effect of oil, since these treatments were mixed in oil.

2. Adaptation of control strategies

2.1. Dry season strategy

The systematic strategy with chlorothalonil induced a fast decrease of the SED (figure 4) and an improvement of the sanitary level in the plantation as shown by the curve of evolution of the YLS (figure 5). At the beginning of the experiment, the YLS was lower in the ‘chlorothalonil’ part (7.3) than in the forecasting strategy plot (9.1). Nevertheless YLS in the ‘chlorothalonil’ part increased regularly all along the experiment and became superior to the YLS observed on the forecasting strategy by the end of March and reached very satisfactory values of 14-15. The SED in the forecasting strategy never decreased significantly and control of the disease was ineffective by the end of the dry season (figure 4). At that time, an important decrease of the YLS has been observed (figure 5). The end of this strategy was then decided, and an application of Sico + Calixin enabled a fast decrease of the SED. A conversion of the forecasting strategy to a systematic use of chlorothalonil prolonged the decrease of the SED and enabled an improvement of the sanitary level (figure 5).

2.2. Rainy season strategy

The systematic strategy with chlorothalonil provided a better control of BLSD in the rainy season than the systematic strategy with penetrants, as shown by the curve of the SED (figure 6). Nevertheless no significant differences were observed in terms of sanitary level as shown by the YLS curve (figure 7). It was decided to stop the penetrant strategy at the beginning of October and, after a transition period of three mancozeb applications in water, to apply a chlorothalonil strategy. During the transition period SED increased significantly and the YLS decreased. Even in the rainy season, the conversion to a chlorothalonil strategy enabled a decrease of the SED and an improvement of the sanitary level (figure 6 & 7).

3. Status of fungicide resistance in the commercial banana plantations of Cameroon

3.1. Resistance to antimitotics

The first resistant strains were detected in 1996 in the commercial banana plantation that is after about 10 years of usage. The frequency of resistant strains was heterogeneous in the first years and became progressively generalised (table 6). This spread of resistance to antimitotics has been observed similarly in all commercial banana plantations (data not shown). Resistant strains were never observed in untreated plantations. In the last monitoring date, there was a trend to a slight decrease of the proportion of resistant strains.

3.2. Resistance to Sterol biosynthesis inhibitors of group 1

In the commercial banana plantation, the mean growth inhibition (GI) was always inferior to the GI measured in the untreated plantations (table 7). By the same, 100 % of the ascospores from the untreated plantation had a GI > 50 %, which was not the case in the commercial plantation where 10-80 % of ascospores had a GI < 50%. So, after some ten years of usage, some sensitivity shifts to propiconazole were observed in the commercial banana plantation. These shifts were subjected to large variations (the mean GI varied from 30 to 70% and the % of ascospores with GI > 50% varied from 17 to 90%) as illustrated by the variation from October 97 to April 98 and from October 2000 to July 2001.

3.3. Resistance to strobilurins

In the untreated plantation, the mean GI was always > 95 %, and the % of susceptible strains, as expressed as those having a GI > 70 % was always 100 %. In the commercial banana plantation, the use of strobilurins started in 2000 and the appearance of resistant strains was very fast by mid of the year 2004 (table 8). However, after a strong increase in the frequency of resistant strains observed successively in December 2004 and June 2005, there was a trend to an important decrease in November 2005.

Discussion

The bio-fungicides that have been experimented have a moderate curative effect because their efficacy would be, in the better case, comparable with that of straight oil or Siganex. This moderate curative effect is not compatible with their use in a forecasting strategy. Nevertheless in a systematic strategy they could be useful in order to reduce the negative environmental effects of BLSD control with more than 40 fungicide applications. Further work is then needed to determine whether Serenade or Natamycin (or any new bio-fungicide) could be used in water alone in a systematic strategy. Effectively, in our trials these fungicides were associated with oil what is not compatible with a systematic strategy. At last, essential oil of *Melaleuca alternifolia* is not suitable for BLSD control, even in a systematic strategy, because of its phytotoxicity and its low effect in water alone.

The experiment on strategies is an illustration of the importance of a high curative effect of fungicides for the success of a forecasting control strategy (de Lapeyre de Bellaire *et al*., 2000). A forecasting strategy with penetrants has not proved to be efficient for BLSD control, even during the dry season. Nevertheless, the level of the disease was elevated at the beginning of the experiment and it would be interesting to experiment again a forecasting strategy starting the experiment with a low disease level (low SED, no leaf spotting i.e; YLS > 14). It is of interest to note that a systematic strategy with chlorothalonil enabled a perfect control of the disease, even during the rainy season, and that a curative effect could be observed after 5-8 weeks. A systematic strategy using penetrants does not provide a better control of BLSD than chlorothalonil in the rainy season, because the SED reduction with this strategy is not as important as the systematic use of chlorothalonil.

New strategies will be experimented in the next future in order to reduce the proportion of chlorothalonil in systematic strategies relying on contact fungicides. In such strategies, chlorothalonil would be used rationally until a low disease level is achieved in the plantation (low SED, no leaf spotting i.e; YLS > 14). Then, other contact fungicides (mancozeb or bio-fungicides) would be applied in water until an increase of SED is observed in the plantation; a new conversion to a chlorothalonil strategy would then be applied. In those strategies (i) fungicides would be applied in water alone in order to enable a rapid conversion to chlorothalonil since this fungicide is very phytotoxic in oil; (2) bio-fungicides could be very useful to reduce the environmental pressure because chlorothalonil is not the best partner for that purpose.

Because a strong curative effect is indispensable to apply a forecasting strategy, this strategy relies essentially on the use of systemic fungicides, and then on the sensitivity of *M. fijiensis* populations to these fungicides (de Lapeyre de Bellaire *et al.*, 2000). New strategies should be developed in order to manage the spatio-temporal development of fungicide resistance. Finally, it is hoped that these strategies would enable to re-introduce systemic fungicides and the use of a rational control strategy based on a forecasting system.

Effectively, fluctuations in the levels of fungicide resistance are regularly observed in the regular monitoring realized in the commercial banana plantations. The hypothesis put forward is that these fluctuations in the frequency of fungicide resistance could result from a lower fitness of resistant strains, which could be counter- selected in the absence of fungicide, and/or gene flow between the treated and the untreated zones. A better knowledge of the parameters implied in fungicide resistance dissemination inside *M. fijiensis* populations (mutation, recombination, genetic drift, gene flow, selection pressure, population size, competition, fitness) is necessary to adapt these strategies.

Then, future work will focus on:

- Gene flow and dispersion of resistance between plantations. Particular attention will be paid to gene-flow between untreated and untreated plantations.
- Measuring the selection or counter – selection through the usage or non-usage of systemic fungicide.
- Evaluation of fitness of susceptible and resistant strains in artificial inoculations.

Acknowledgement. The authors wish to thank the banana producers of Cameroon for their strong implication in the work presented here. The society DSM food, Agraquest and Biomor for supporting work on the bio-fungicides Natamycin, Serenade and Timorex. F. Tchipé, O. Nguidjo, R. Dongmo, J. Essome and S. Kana for technical assistance.

References

1. Amil, A., Cook, A., Stanger, C., Knight, S., Wirz, M., Shaw, M. 2002. Las dinamicas de la sensibilidad de *Mycosphaerella fijiensis* a estrobilurinas (QoI's) en Costa Rica. In Proceedings of XVth Reunion of the ACORBAT. Medellin, Colombia, 27 oct-2 nov 2002. p. 158- 162.

2. Bureau E. 1990. Adaptation d'un système d'avertissement à la lutte contre la cercosporiose noire (*Mycosphaerella fijiensis* Morelet) en plantation de banane plantain au Panama. Fruits, 45 : 329-338.

3. de Lapeyre de Bellaire L., Mourichon X., Ganry J., 2000. Forecasting less fungicide for Sigatoka disease in Guadeloupe. Biocontrol News and Information, 21, (3), p. 68N-69N.

4. Fouré, E. (1982) Les cercosporioses du bananier et leurs traitements. Comportement des variétés. Etudes de la sensibilité variétale des bananiers et plantains à *Mycosphaerella* f*ijiensis* Morelet au Gabon (Maladie des Raies Noires). I : Incubation et évolution de la maladie. Fruits 37 (12), 749–759.

5. Fouré E. 1984. Etude de la sensibilité variétale des bananiers et des plantains à *M. fijiensis* Morelet et de quelques caractéristiques biologiques de la maladie au Gabon. Fruits, 39 : 365-378.

6. Fouré E. 1988. Stratégies de lutte contre la cercosporiose noire des bananiers et plantains provoquée par *Mycosphaerella fijiensis* Morelet. L'avertissement biologique au Cameroun. Evaluation des possibilités d'évaluation. Fruits, 43 : 269-274.

7. Fouré E., Mouliom-Pefoura A., Mourichon X. 1990. Etude de la sensibilité variétale des bananiers et des plantains à *M. fijiensis* Morelet au Cameroun. Caractérisation de la résistance au champ de bananiers appartenant à divers groupes génétiques. Fruits : 45, 339-345.

8. Fouré E., Moreau A. 1992. Contribution à l'étude épidémiologique de la cercosporiose noire dans la zone de Moungo au Cameroun de 1987 à 1989. Fruits: 47, 3-16.

9. Marin, D.H., Romero R.A., Guzman M., Sutton T.B. 2003. Black Sigatoka: an increasing threat to banana cultivation. Plant Disease: 87, 208-222.

10. Pasberg-Gauhl C., F. Gauhl & D.R. Jones. 2000. Black leaf streak. Distribution and economic importance. *In* Jones D.R. ed. Diseases of Bananas, Abaca and Enset. Wallingford. UK, CABI, p. 37-44.

11. Sierotzki, H. , Parisi, S., Steinfeld, U., Tenzer, I., Poirey, S., Gisi, U. 2000. Mode of resistance to respiration inhibitors at the cytochrome bc1 enzyme complex of *Mycosphaerella fijiensis* field isolates. Pest Management Science, 56: 833-841.

12. Stover R.H.& J.D. Dickson 1970. Leaf spot of banana caused by *Mycosphaerella musicola:* methods of measuring spotting prevalence and severity. Tropical Agriculture *(Trinidad)* 47: 289-302.

13. Stover, R.H. 1974. Effect of measured levels of Sigatoka disease of bananas on fruit quality and leaf senescence. Tropical agriculture, 51: 531-542.

Table 1. Presentation of the different fungicide treatments compared in the course of 3 experiments conducted in CARBAP experimental station in Njombe, for the control of BLSD

Experiment	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5
1. February to April 2005	Timorex (2L) in water	Timorex (2L) in oil	Siganex (0,5 L) in emulsion		
2. January to April 2005	Natamycin (100g) in oil	Siganex (0,5 L) in emulsion	Untreated control		
3. June to December 2005	Serenade (2L) in oil	Straight oil	Serenade (1L) + Dithane F488 (1.5 L) in oil	Dithane F488 (3 L) in oil	Untreated control

Table 2. Coefficients attributed to the different (leaf number) / (stage of the disease) association, for the calculation of the Stage of Evolution of the Disease (SED)

Stage of the disease[x]		Leaf II	Leaf III	Leaf IV
1	-	60	40	20
	+	100	80	60
2	-	100	80	60
	+	140	120	100
3	-	140	120	100
	+	180	160	140
4	-	180	160	140
	+	200	200	180
5	-	220	200	180
	+	260	240	220
6	-	260	240	220
	+	300	280	260

[x] for each stage of the disease, the coefficient attributed depends on density of symptoms: (-) if less than 50 lesions are observed on the leaf ; (+) if more than 50 lesions are observed on the leaf

Table 3. Efficacy of essential oil of *Melaleuca alternifolia* for the control of BLSD. SED and YLS were assessed every week from February 3rd to April 7th 2005. All treatments were applied every week with a hand sack atomizer, in 20L/ha. The mean of 3 replicates per treatment were submitted to an ANOVA.

Treatments	SED[x]	YLS[x]
Timorex (2L) in water	1152 (b)	6.4 (b)
Timorex (2L) in oil	747 (a)	7.5 (a)
Siganex (0,5 L) in an emulsion	512 (a)	7.9 (a)

[x] Values followed by the same letter do not differ at the 5% probability level, using the Tukey's test

Table 4. Efficacy of Natamycin for the control of BLSD. SED and YLS were assessed every week from January 1st to April 4th 2005. All treatments were applied every week with a hand sack atomizer in 20L/ha. The mean of 3 replicates per treatment were submitted to an ANOVA.

Treatments	SED[x]	YLS[x]
Natamycin (100g) in oil	1267 (a)	7.2 (a)
Siganex (0,5 L) in an emulsion	1317 (a)	7.3 (a)
Untreated control	2261 (b)	5.9 (b)

[x] Values followed by the same letter do not differ at the 5% probability level, using the Tukey's test

Table 5. Efficacy of *Bacillus subtilis* for the control of BLSD. SED and YLS were assessed every week from June 6th to December 9th 2005. All treatments were applied every week with a hand sack atomizer in 20L/ha. The mean of 3 replicates per treatment were submitted to an ANOVA.

Treatments	SED[x]	YLS[x]
Serenade (2L) in oil	2446 (b)	5.2 (a)
Straight oil	2437 (b)	5.4 (a)
Serenade (1L) + Dithane F488 (1.5 L) + oil	2158 (a)	5.4 (a)
Dithane F488 (3 L) + oil	2297 (ab)	5.4 (a)
Untreated control	3207 (c)	4.6 (b)

[x] Values followed by the same letter do not differ at the 5% probability level, using the Tukey's test

Table 6. Evolution of fungicide resistance to benomyl or methyl-thiophanate (Benlate or Callis) in a commercial banana plantation located in Njombe. Resistant ascospores had a normal germination on agar amended with 5 ppm benomyl or methyl-thiophanate, since susceptible strains did not germinate or had a distorted germ tube.

Years	% Resistant strains in the commercial plantation (Min-Max)	% Resistant strains in the untreated plantation
1993	0	0
1994	0	0
1995	0	0
1996	15 (0-60)	0
1997	22	0
1998	46	0
2000	18 (14-21)	0

2002	52 (33-71)	0
2003	56	0
2004	37	0

Table 7. Evolution of fungicide resistance to propiconazole (Tilt) in a commercial banana plantation located in Njombe. Growth inhibition (GI) was evaluated by comparison of germ tube length of ascospores germinating on agar amended with 0.1 ppm propiconazole and on agar alone as control.

Date	Mean GI in the commercial plantation	Mean GI in an untreated plantation	% ascospores with GI > 50% in the commercial plantation	% ascospores with GI > 50% in an untreated plantation
10/1996	61	-	-	-
05/1997	61	-	-	-
10/1997	70	-	-	-
04/1998	54	72	56	-
01/1999	49	-	50	-
01/2000	61	73	80	-
10/2000	61	73	90	100
07/2001	30	78	17	100
01/2002	56	76	60	100
01/2003	49	74	63	100
09/2003	52	72	30	100
05/2004	54	83	52	100
01/2005	61	84	72	100

Table 8. Evolution of fungicide resistance to azoxystrobine (Bankit) in a commercial banana plantation located in Mbanga. Growth inhibition was evaluated by comparison of germ tube length of ascospores germinating on agar amended with 1 ppm azoxystrobine and on agar alone as control.

Date	Mean GI in the commercial plantation	Mean GI in an untreated plantation	% ascospores with GI > 70% in the commercial plantation	% ascospores with GI > 70% in an untreated plantation
08/2001	90	100	100	100
09/2003	96	98	100	100
04/2004	86	95	74	100
10/2004	34	98	2	100
06/2005	41	97	23	100
11/2005	81	96	73	100

Figure 1. Evolution of BLSD in a banana plantation of Cameroon, in 1990. In this plantation, timing of fungicide treatments has been decided according to the Stage of Evolution of the Disease (SED). Fungicide applications are represented by arrows: antimitotics (red) and triazoles (black).

Figure 2. Evolution of chemical control of BLSD in the banana plantations of Cameroon. The average number of annual treatments, as well as the proportion of the different groups of fungicides is indicated: systemic fungicides (antimitotics, Sterol Biosynthesis Inhibitors of group 1, strobilurins); penetrants (Sterol Biosynthesis Inhibitors of group 2, pyrimethanil); contact fungicides (mancozeb, chlorothalonil).

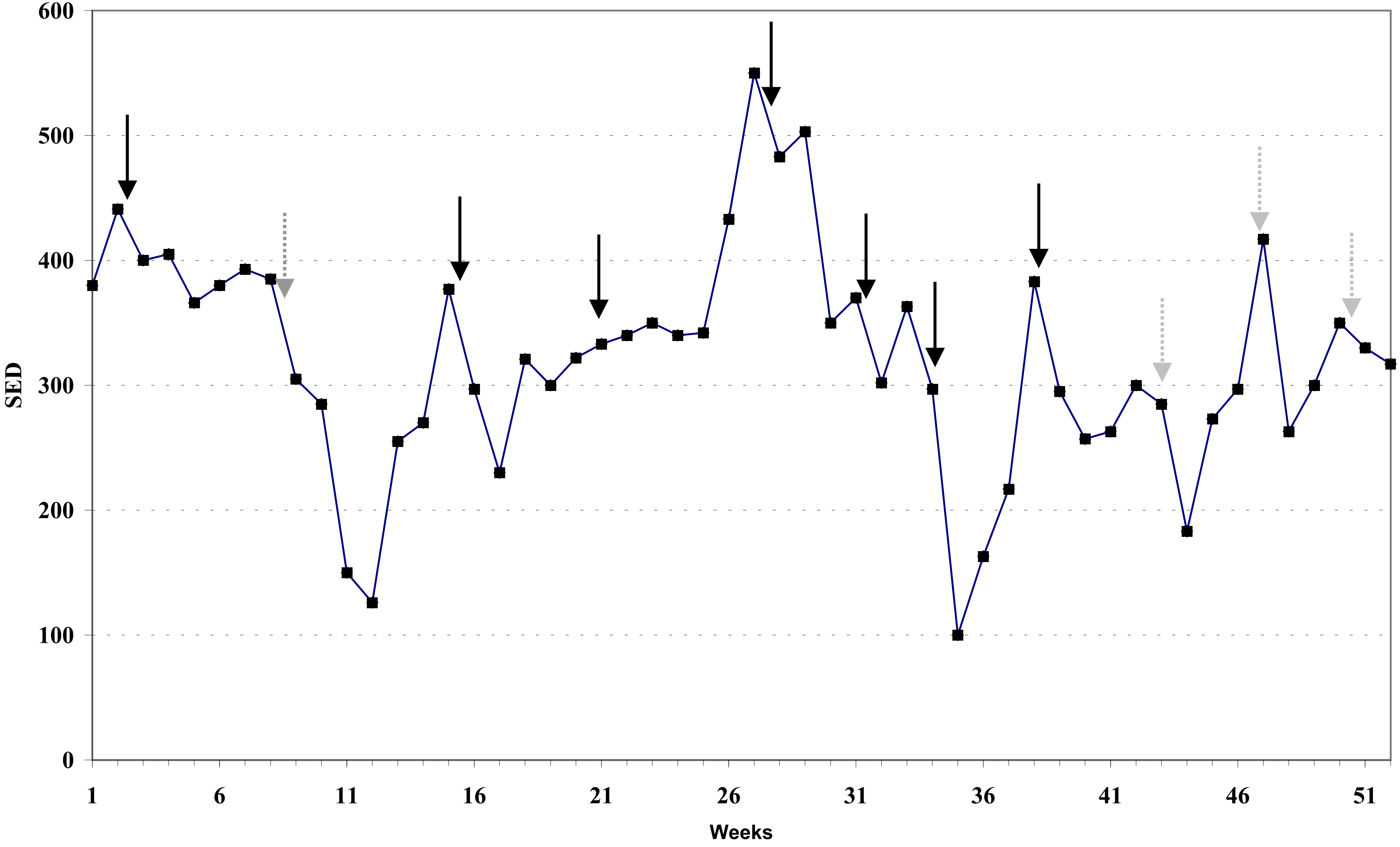
Figure 3. Weekly rainfall observed in the commercial banana plantation selected for the experiments of new control strategies, over a 1 year period. Rainy season extends from April to November.

Figure 4. Evolution of the SED in the course of an experiment where a forecasting strategy with penetrants (blue line) has been compared with a systematic use of chlorothalonil (red line), during the dry season. Fungicide application are represented by arrows: Siganex (Sg); Sterol Biosynthesis Inhibitors of group 2 (I2); Sterol Biosynthesis Inhibitors of group 1 (I1); Chlorothalonil (green arrows, dot line).

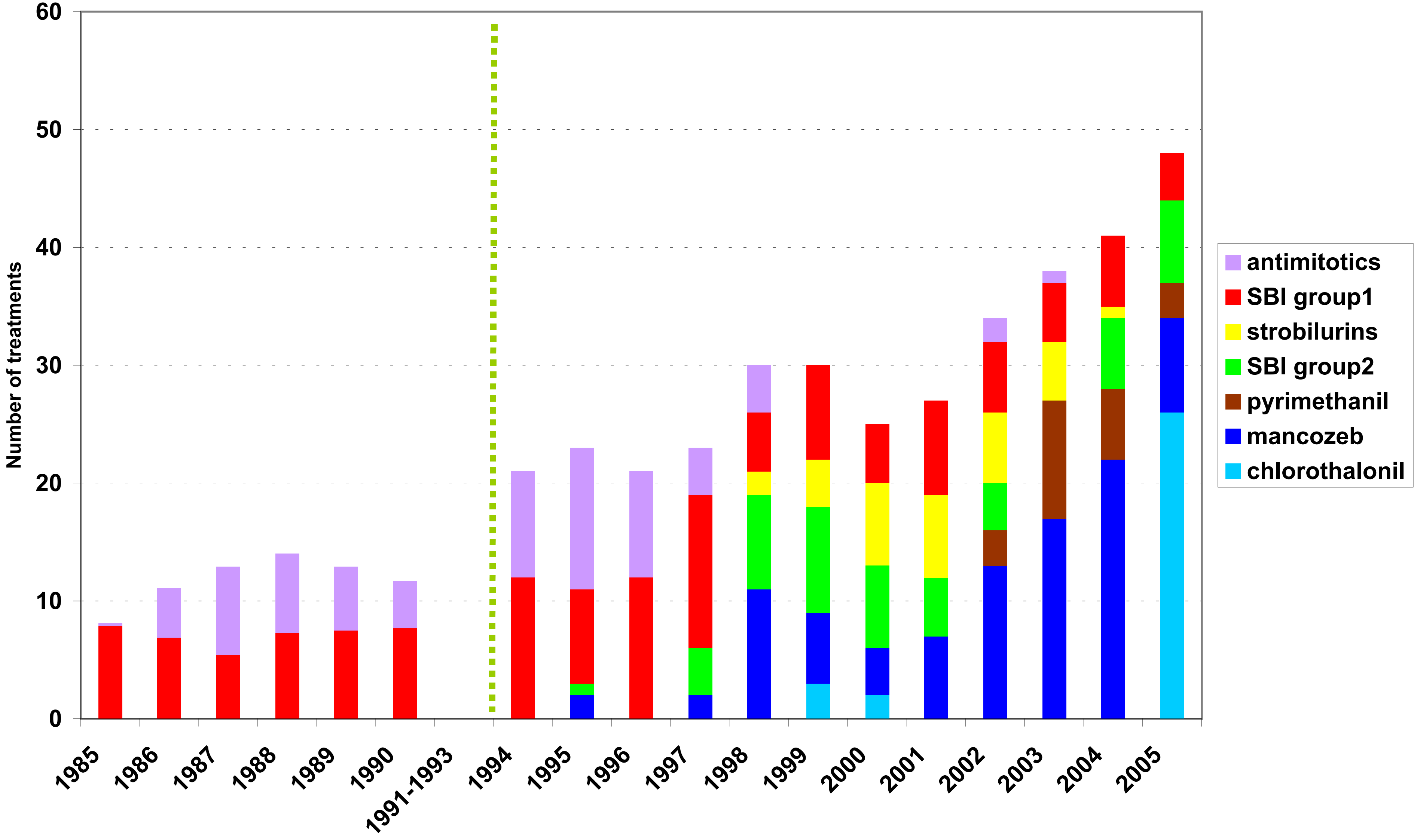
Figure 5. Evolution of the YLS in the course of an experiment where a forecasting strategy with penetrants (blue line) has been compared with a systematic use of chlorothalonil (red line), during the dry season.

Figure 6. Evolution of the SED in the course of an experiment where a systematic use of penetrants (blue line) has been compared with a systematic use of chlorothalonil (red line), during the rainy season. Fungicide application are represented by arrows: Siganex (Sg); Sterol Biosynthesis Inhibitors of group 2 (I2); Penncozeb (p); Chlorothalonil (green arrows, dot line). Penetrants have been applied on a 10 day timetable and chlorothalonil on a 7 days timetable.

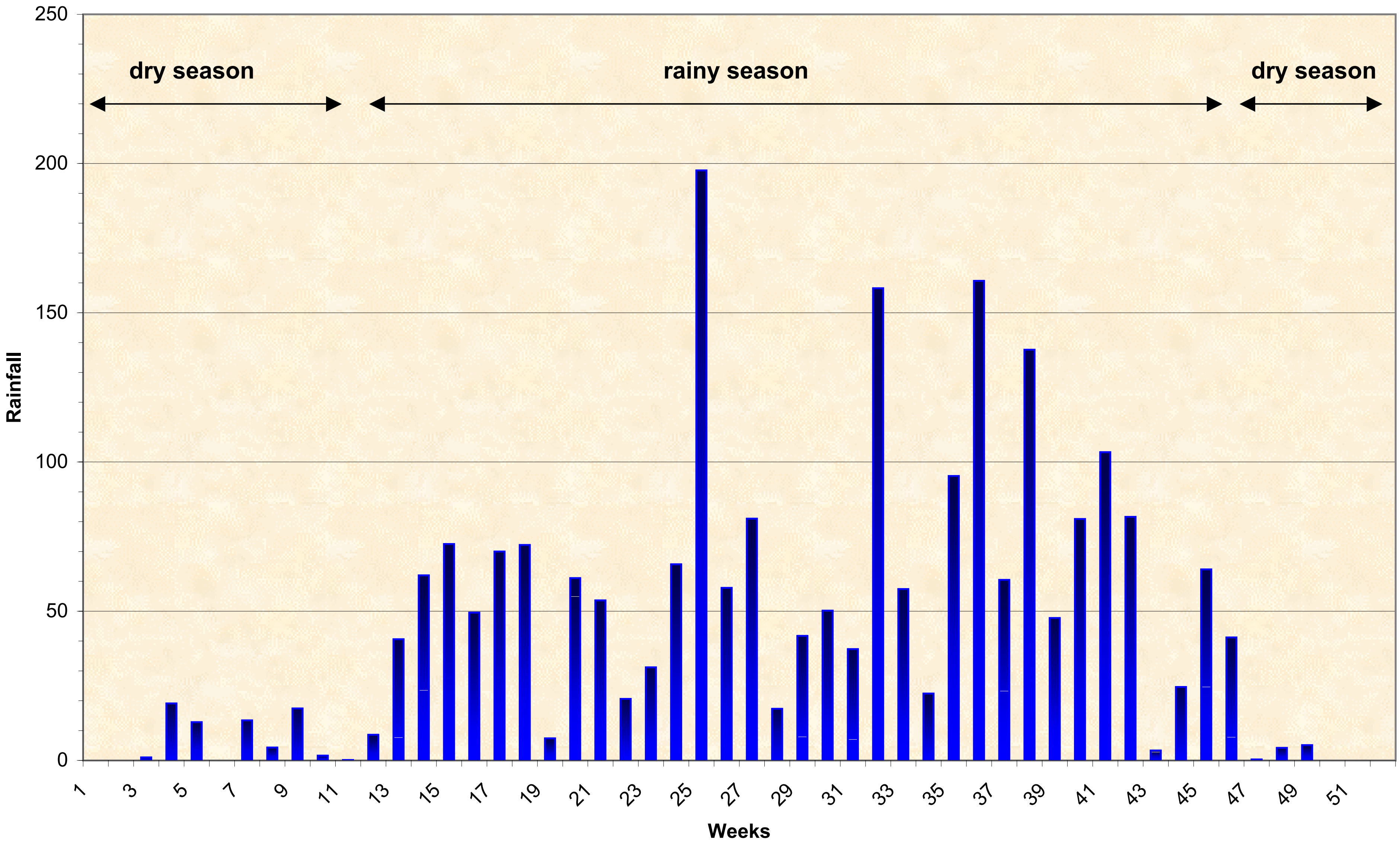
Figure 7. Evolution of the YLS in the course of an experiment where a systematic use of penetrants (blue line) has been compared with a systematic use of chlorothalonil (red line), during the rainy season. Penetrants have been applied on a 10 day timetable and chlorothalonil on a 7 days timetable.



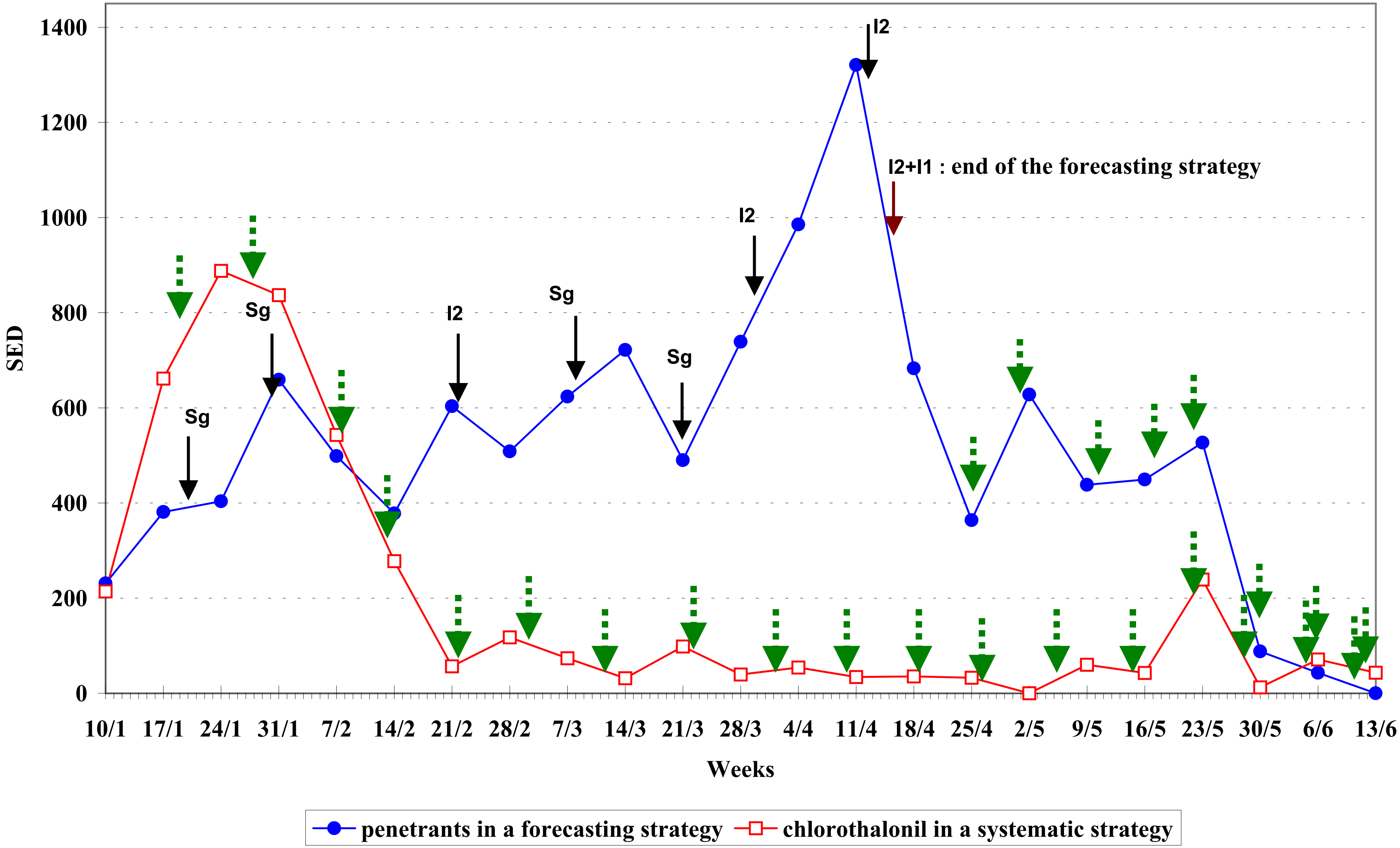
600
500
400
300
200
100
0
SED
1
6
11
16
21
26
31
36
41
46
51
Weeks



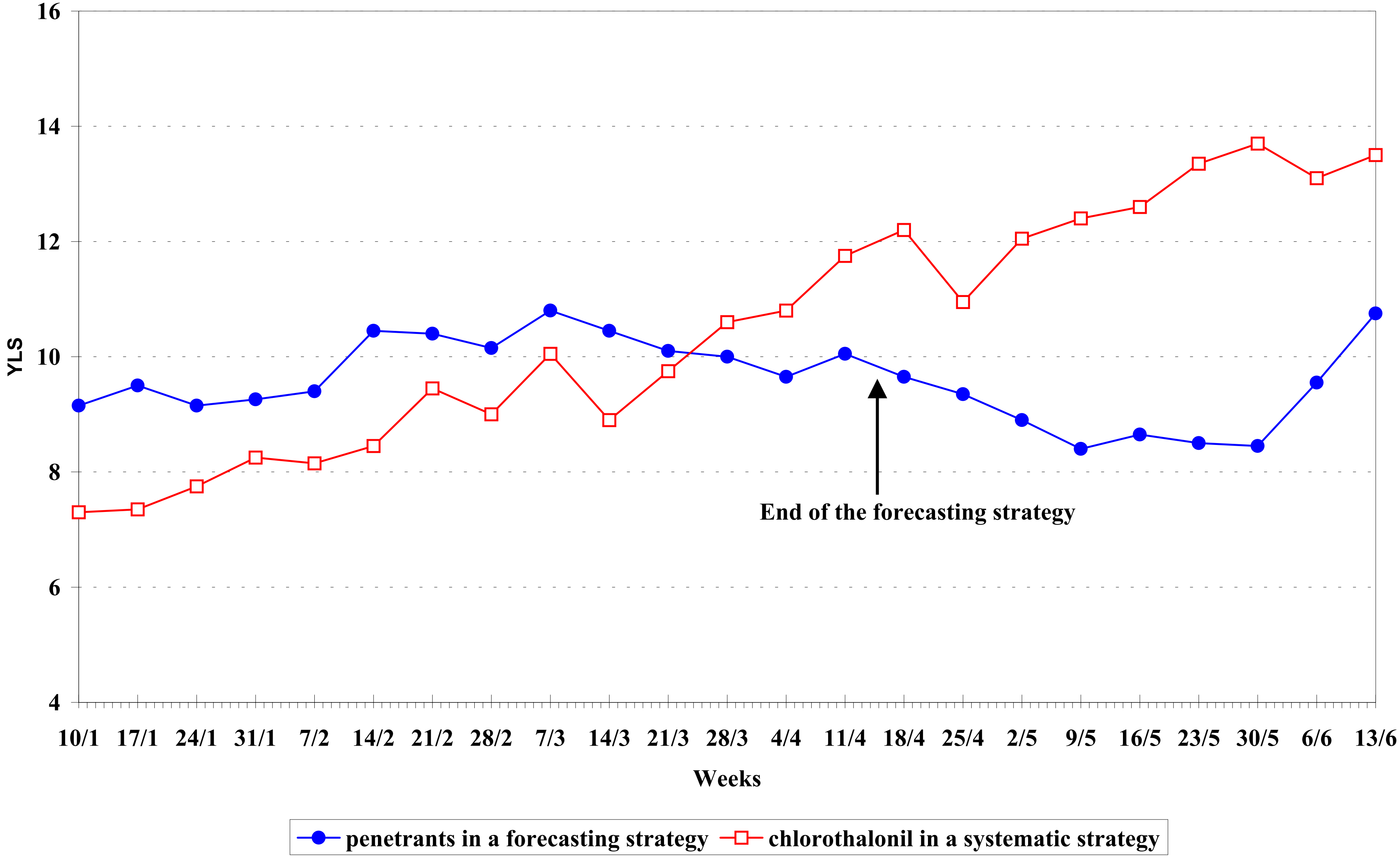
60
50
40
30
20
10
0
Number of treatments
1985
1986
1987
1988
1989
1990
1991-1993
1994
1995
1996
1997
1998
1999
2000
2001
2002
2003
2004
2005
antimitotics
SBI group1
strobilurins
SBI group2
pyrimethanil
mancozeb
chlorothalonil

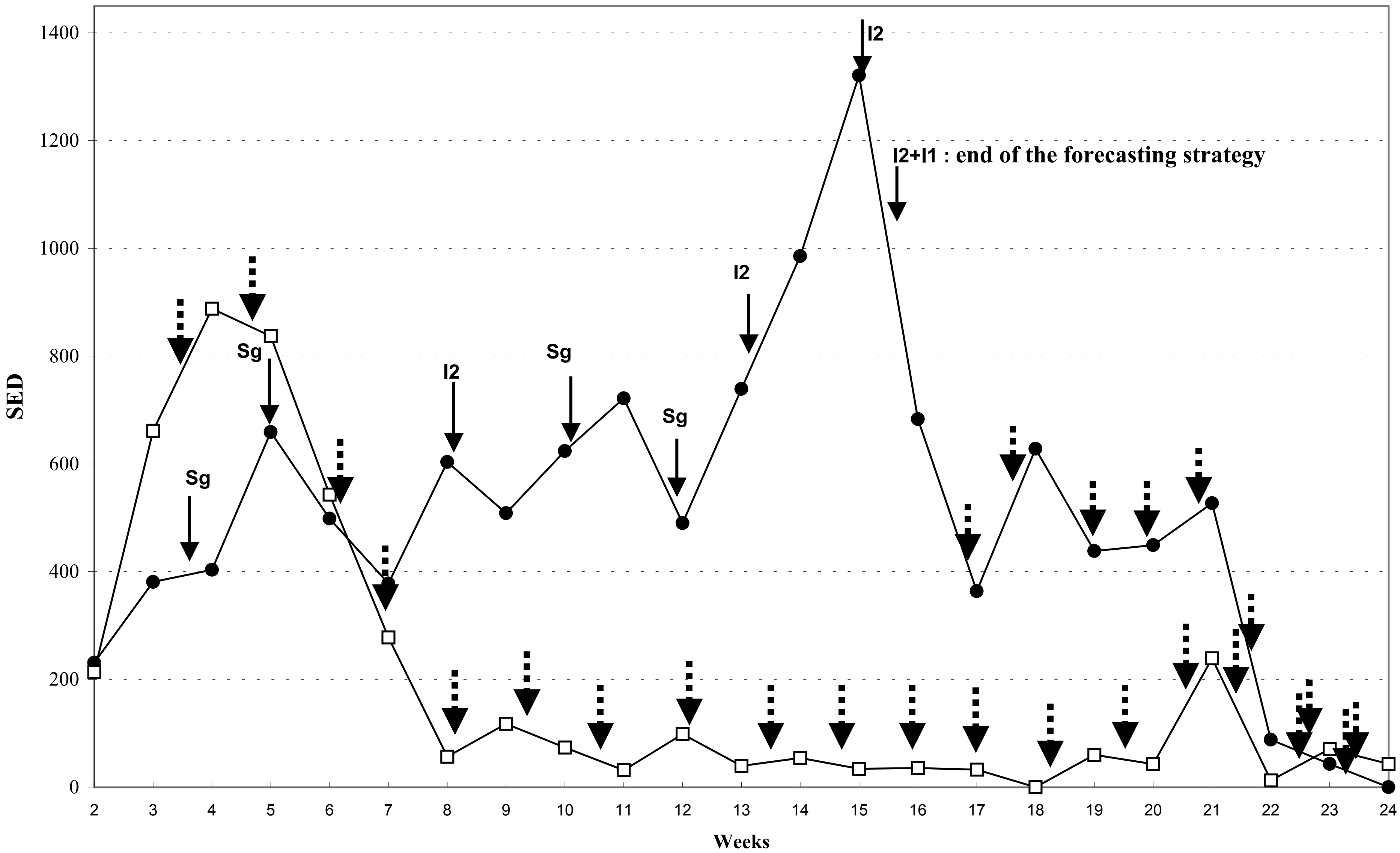


dry season
rainy season
dry season
Rainfall
250
200
150
100
50
0
1
3
5
7
9
11
13
15
17
19
21
23
25
27
29
31
33
35
37
39
41
43
45
47
49
51
Weeks

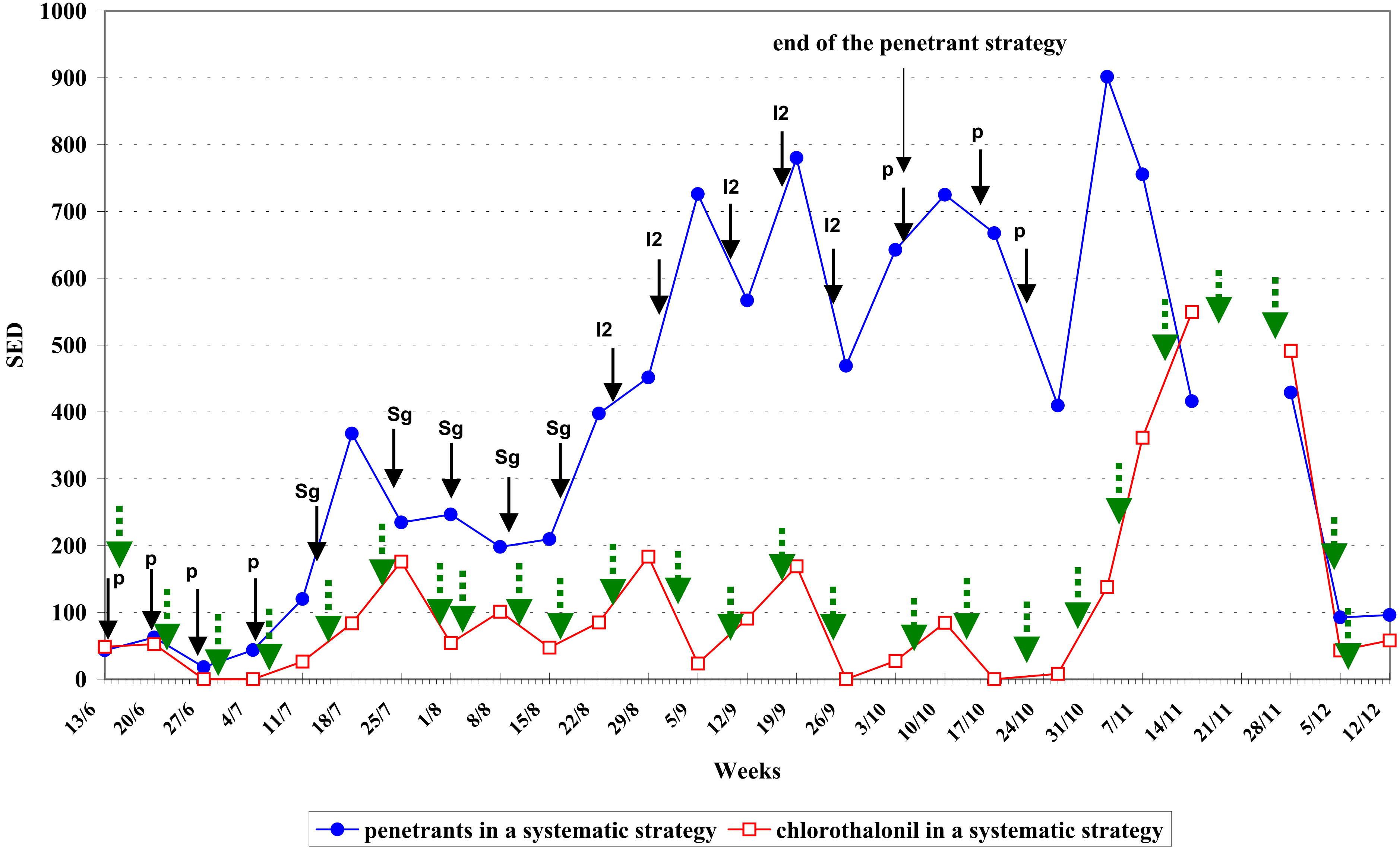


SED
1400
1200
1000
800
600
400
200
0
Sg
Sg
I2
Sg
Sg
I2
I2
I2+I1 : end of the forecasting strategy
10/1
17/1
24/1
31/1
7/2
14/2
21/2
28/2
7/3
14/3
21/3
28/3
4/4
11/4
18/4
25/4
2/5
9/5
16/5
23/5
30/5
6/6
13/6
Weeks
penetrants in a forecasting strategy
chlorothalonil in a systematic strategy

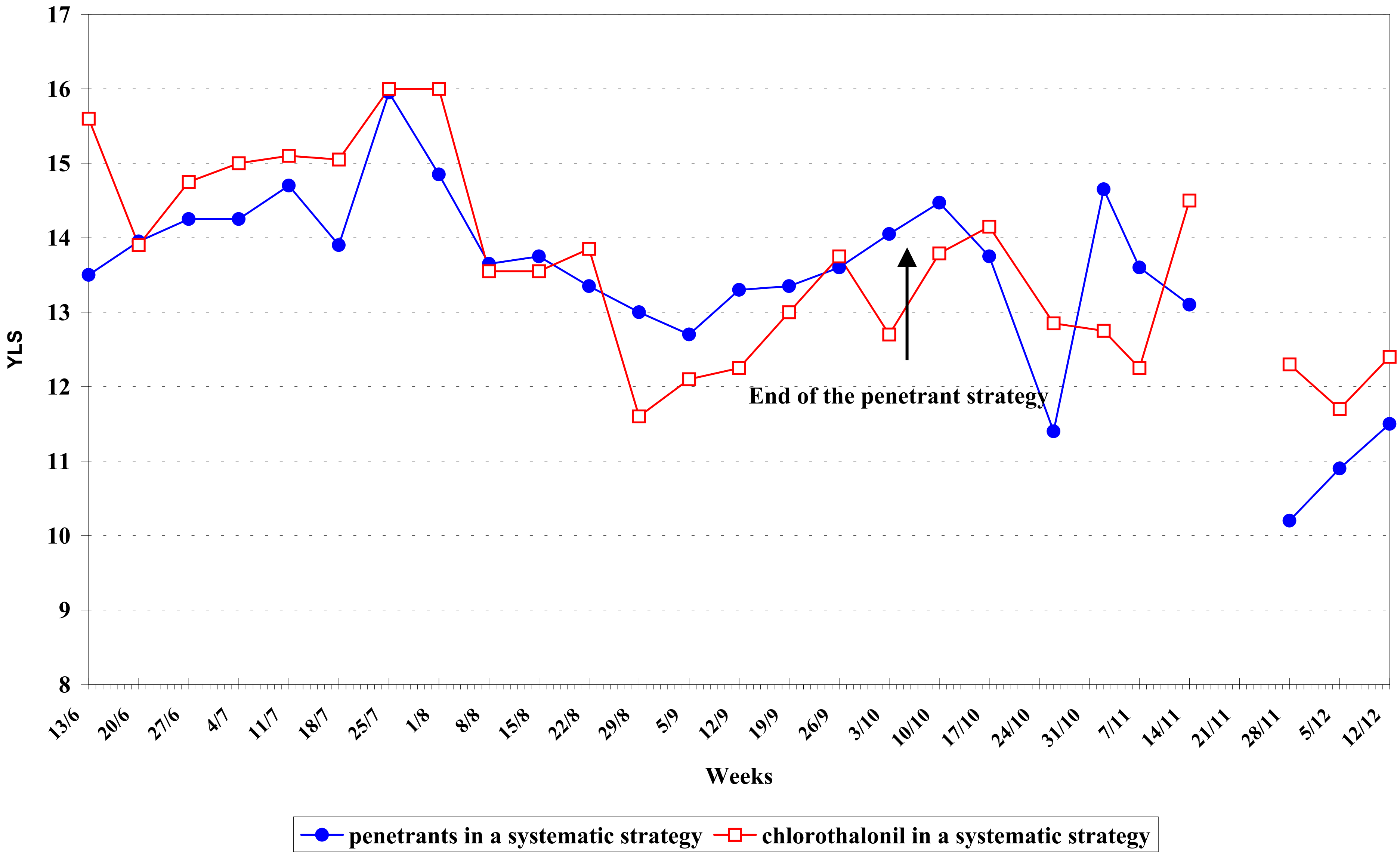

16
14
12
10
8
6
4
YLS
End of the forecasting strategy
10/1
17/1
24/1
31/1
7/2
14/2
21/2
28/2
7/3
14/3
21/3
28/3
4/4
11/4
18/4
25/4
2/5
9/5
16/5
23/5
30/5
6/6
13/6
Weeks
penetrants in a forecasting strategy
chlorothalonil in a systematic strategy



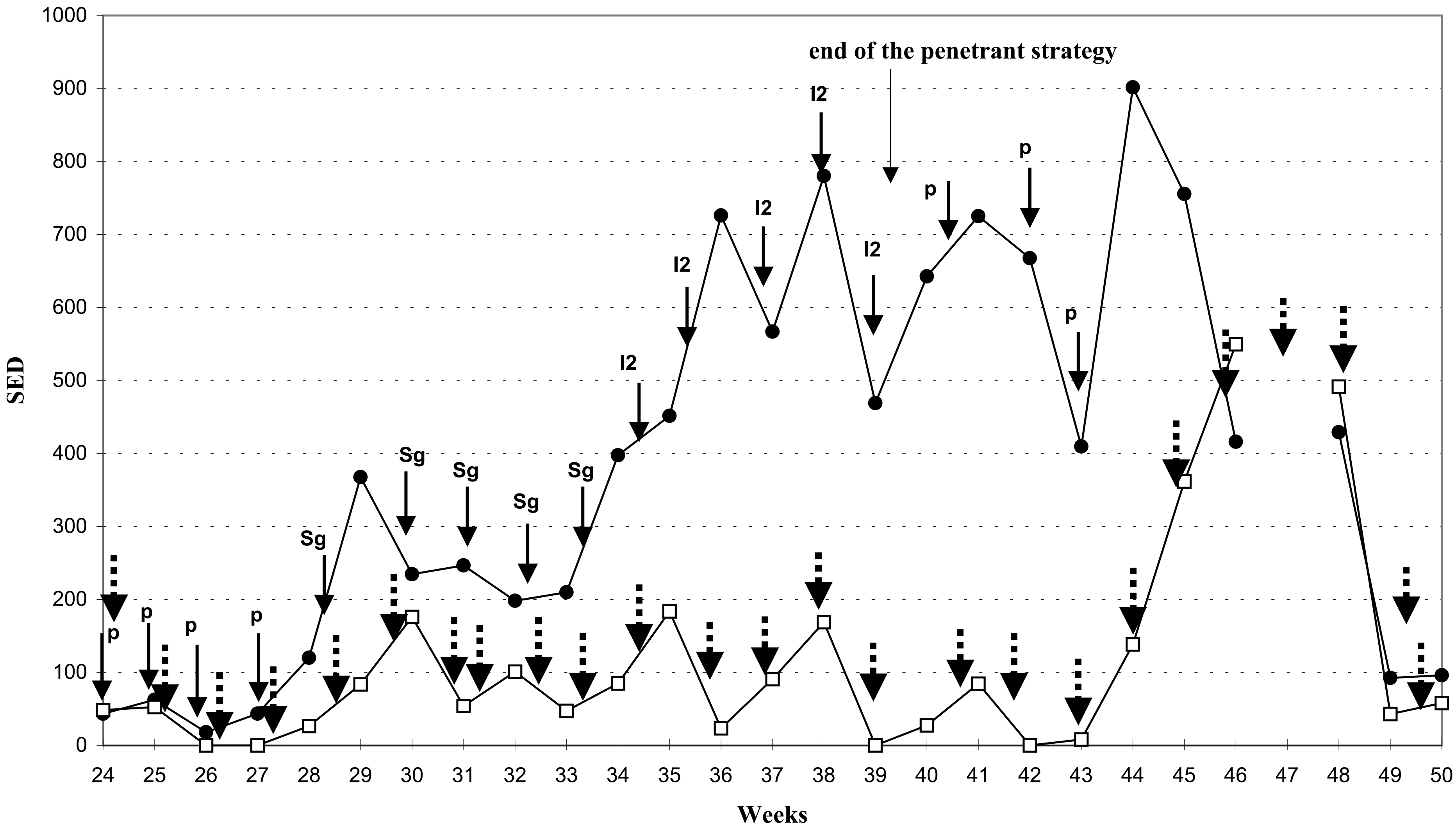
SED
Weeks
1400
1200
1000
800
600
400
200
0
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
Sg
Sg
I2
Sg
Sg
I2
I2
I2+I1 : end of the forecasting strategy



end of the penetrant strategy
SED
Weeks
1000
900
800
700
600
500
400
300
200
100
0
13/6
20/6
27/6
4/7
11/7
18/7
25/7
1/8
8/8
15/8
22/8
29/8
5/9
12/9
19/9
26/9
3/10
10/10
17/10
24/10
31/10
7/11
14/11
21/11
28/11
5/12
12/12
p
Sg
I2
penetrants in a systematic strategy
chlorothalonil in a systematic strategy



17
16
15
14
13
12
11
10
9
8
YLS
End of the penetrant strategy
13/6
20/6
27/6
4/7
11/7
18/7
25/7
1/8
8/8
15/8
22/8
29/8
5/9
12/9
19/9
26/9
3/10
10/10
17/10
24/10
31/10
7/11
14/11
21/11
28/11
5/12
12/12
Weeks
penetrants in a systematic strategy
chlorothalonil in a systematic strategy



end of the penetrant strategy
SED
Weeks
p
Sg
l2
1000
900
800
700
600
500
400
300
200
100
0
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50