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Effects of SPLAT® GM sprayable pheromone formulation on gypsy moth mating success

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Abstract

Several integrated pest management programs rely on the use of mating disruption tactics to control insect pests. Some programs specifically target non-native species, such as the gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae). We evaluated SPLAT® GM, a new sprayable formulation of the gypsy moth sex pheromone disparlure, for its ability to disrupt gypsy moth mating. The study was conducted in 2006, 2007, and 2008 in forested areas in Virginia, USA. Mating success of gypsy moth females was reduced by >99% and male moth catches in pheromone-baited traps by >90%, in plots treated with SPLAT® GM at dosages ranging from 15 to 75 g of active ingredient (a.i.) ha^{-1}. Dosage-response tests conducted in 2008 indicated that SPLAT® GM applied at a dosage of 7.5 g a.i. ha^{-1} was as effective as a 15 g a.i. ha^{-1} dosage.

Introduction

Mating disruption is a technique in which synthetic pheromone is applied to disrupt mating communication, and a strategy used in management programs for many insect pests (Cardé & Minks, 1995; Howse et al., 1998; Yamanaka, 2007; Witzgall et al., 2008), including gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae) (Thorpe et al., 2006). Compared to other management tactics and especially those involving chemical insecticides, mating disruption tends to be less expensive, more environmentally-friendly, and associated with fewer non-target effects. The gypsy moth is one of the most economically important forest pests in the eastern USA. Larvae can exploit >300 species of trees in most climatic zones in the USA (Liebhold et al., 1995; Gray, 2004). In addition to forest and shade trees, gypsy moth also poses a threat to a number of fruit and nut crops such as apple, apricot, blueberry, filbert, pear, pistachio, and plum (Miller et al., 1987). In addition to mating disruption, primary control methods include biopesticides, such as *Bacillus thuringiensis* var. *kurstaki* (Btk) and the gypsy moth nucleopolyhedrosis virus (registered as Gypchek®), and the insect growth regulator diflubenzuron (registered as Dimilin®) (Tobin & Blackburn, 2007). In the gypsy moth 'Slow the Spread' program (STS), over 200 000 hectare per year are managed using mating disruption (Tobin & Blackburn, 2007; Gypsy Moth Digest, 2009).

Extensive research has been conducted to optimize the mating disruption technique against the gypsy moth (e.g., Thorpe et al., 2006; Onufrieva (Tcheslavskaia) et al., 2008; Onufrieva et al., 2008). Yet, despite this effort, Disrupt® II (Hercon Environmental, Emigsville, PA, USA), a plastic flake formulation of disparlure, is the only registered gypsy moth mating disruption product for use in the STS program (USDA, 1995; Thorpe et al., 2006). The optimal dosage for Hercon Disurpt® II was determined to be 15 g active ingredient (a.i.) ha^{-1}, whereas a dosage of 37.5 g a.i. ha^{-1} is sometimes used in areas with higher population density and lower summer temperatures (Webb et al., 1990; Tcheslavskaia et al., 2005; Onufrieva (Tcheslavskaia) et al., 2008).

Because of the importance of mating disruption tactics in gypsy moth management programs, and concerns of non-target effects and environmental contamination as

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the gypsy moth invades or is introduced into new areas, there is a need to consider alternative formulations to improve cost-effectiveness while achieving management goals. In this paper, we present the results of the evaluations of a new formulation of disparlure, SPLAT® GM (ISCA Technologies, Riverside, CA, USA). We conducted field experiments in 2006, 2007, and 2008 to determine its efficacy in disrupting mating in gypsy moth populations as measured by the mating success of deployed females and the number of male moths caught in deployed pheromone-baited traps.

Materials and methods

We evaluated the two formulations of disparlure, Hercon Disrupt® II and SPLAT® GM. We conducted our experiments in the Appomattox-Buckingham (ABSF) and Cumberland (CFS) State Forests, VA, USA (UTM 746246 E, 4166292 N to 700180 E, 4136389 N, NAD 27, zone 17), in Goshen Wildlife Management Area (GWMA) [Bath County, VA, USA (UTM 637052 E, 4223294 N to 614250 E, 4192715 N, NAD 27, zone 17)] in 2007, and in GWMA and Rockbridge County, VA, USA in 2008 (UTM 632723 E, 4199588 N to 632432 E, 4200432 N, NAD27, zone 17).

Plot layout and pheromone treatments

Field test 2006. Ten plots, each 500 × 500 m and separated by at least 1 km, were selected. The plots were grouped into two blocks with five plots per block. In each block, one plot was used as a control and left untreated, and the remaining four plots were treated as follows: Disrupt® II at 15 g a.i. ha^{-1}, Disrupt® II at 37.5 g a.i. ha^{-1}, SPLAT® at 15 g a.i. ha^{-1}, and SPLAT at 37.5 g a.i. ha^{-1}. Due to application problems only one of the plots treated with Disrupt® II at 37.5 g a.i. ha^{-1} was used for the analysis, whereas the rest of the treatments were replicated twice. Plots treated with Disrupt® II were monitored from June 22 to August 8. Due to formulation issues, SPLAT® GM was applied 3 weeks later and the plots were monitored from 13 July to 8 August. Additional release of marked laboratory-reared males was done in control plots and plots treated with SPLAT® GM at 15 and 37.5 g a.i. ha^{-1} on 19 September which is 11 weeks after SPLAT® GM was applied.

Field test 2007. We used six 500 × 500 m study plots separated by at least 1 km that were grouped into two blocks with three plots per block. In each block, one plot was left untreated and used as control; one plot was treated with Hercon Disrupt® II at 15 g a.i ha^{-1} for use as positive control, and one plot was treated with SPLAT® GM. In one of the two blocks, SPLAT® GM was applied at 15 g a.i. ha^{-1}; however, in the second block the plot was treated with SPLAT® GM at an overall approximate rate of about 12 g a.i. ha^{-1}, due to the problems with calibration and nozzle blockage. For the purpose of data analysis, however, we assumed both plots as being treated at the same rate (15 g a.i. ha^{-1}). Plots were monitored for 11 weeks (27 July–27 August).

Field test 2008. We used 14 500 × 500 m study plots separated by at least 1 km that were grouped into two blocks with seven plots per block. All of these plots were in low-density background gypsy moth populations. In each block, one plot was left untreated and used as a control, one plot was treated with Hercon Disrupt® II at 15 g a.i. ha^{-1} and used as a positive control, and the remaining plots were each treated with SPLAT® GM at 0.15, 1.5, 3, 7.5, or 15 g a.i. ha^{-1}. Due to application problems, the 0.15 g a.i. ha^{-1} plot was only done in one plot. Plots were monitored for 8 weeks (16 July–26 August).

Pheromone applications

The Disrupt® II formulation consisted of plastic flakes composed of polyvinyl chloride (PVC) outer layers and an inner polymer layer containing 17.9% racemic disparlure. The flakes were mixed with diatomaceous earth (3% wt/wt) to reduce clogging, and aerially applied using a fixed-wing aircraft (Air Tractor) equipped with specialized application pods (Schweitzer Aircraft, Elmira, NY, USA). Within the pods, the flakes were mixed with a multipolymer emulsion glue (Gelva 2333; Solutia, Springfield, MA, USA) and dispensed through a spinner (Thorpe et al., 2006). Disparlure release rate from applied flakes was not determined in this study. However, in previous studies where plastic flakes were applied under similar conditions, the flakes released 30–50% of their disparlure content over the 6-week period of male moth flight (Leonhardt et al., 1996; Thorpe et al., 1999).

SPLAT® GM is a liquid formulation developed by ISCA Tech (Riverside, CA, USA) that is designed for both aerial and ground application. The formulation contains 13.0% racemic disparlure and is applied with conventional application systems pressurized either by positive displacement pumps, pressurized gas cylinders, or a combination of both. SPLAT® GM was applied using Beechcraft King Air aircraft. Disparlure release rate from applied microcapsules was not determined in this study. A Global Positioning Satellite (GPS) navigation system was used to guide all spray applications.

Treatment evaluation

The efficacy of each treatment in disrupting mating was evaluated by deploying laboratory-reared tethered females following the release of laboratory-reared males. Each

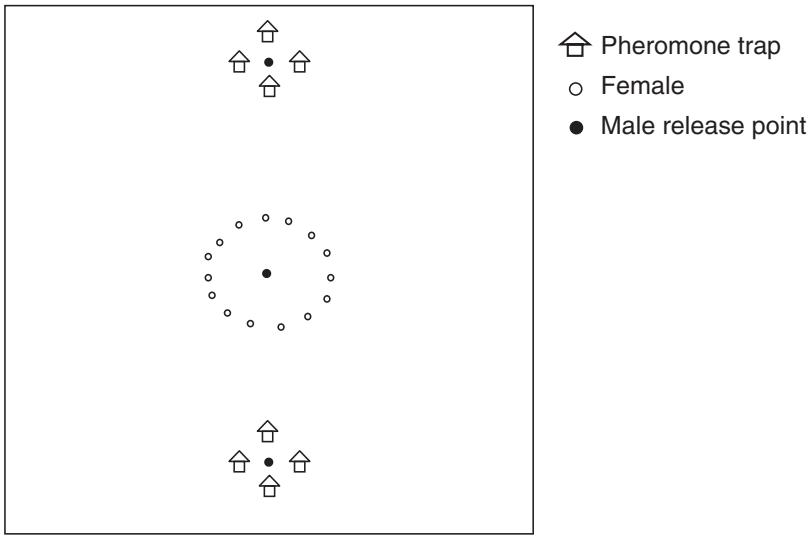

Figure 1 Layout of pheromone-baited traps, male moth release points, and tethered females in an experimental forest plot.

study plot had three male moth release points (Figure 1). Fifteen tethered females were placed in a circle around a release point at the center of the plot. Four pheromone-baited traps were placed around two male release points 150 m to the north and south of the plot center; the traps were positioned 25 m from each release point. Adult females were placed on tree boles for 1 day and protected from predation by a band of Tanglefoot bird repellent (Thorpe et al., 2007). In 2006 and 2007, treatment evaluations were conducted using both males and females. In 2008, only released males were used to evaluate the efficacy of mating disruption. Male and female gypsy moths were obtained as pupae from the USDA Animal and Plant Health Inspection Service, Pest Survey Detection and Exclusion Laboratory, OTIS Air National Guard Base, MA, USA. Pupae were kept in laminated paper cups with plastic lids. A fluorescent dye solvent red 26 (Royce International, Paterson, NJ, USA) was added to the caterpillars' diet at the rearing facility.

Adult virgin gypsy moth females were left on trees for 24 h, after which they were removed and their fertilization status was determined by the eggs' embryonation (Stark et al., 1974; Sharov et al., 1995; Tcheslavskaia et al., 2002). Male moth recapture was determined using standard USDA milk-carton pheromone traps baited with 500 μg of (+)-disparlure in twine dispensers (Hercon Environmental, Emigsville, PA, USA) (Schwalbe, 1981; Leonhardt et al., 1992). Each week, ca. 150 adult males were released at each release point (Figure 1). Pheromone-baited traps were checked and emptied at the time of release. Male moths captured in pheromone-baited traps were removed and stored in the freezer. The moths were later examined under the microscope with UV light for the presence of fluorescent powder to distinguish between released and native moths. Only laboratory-reared, released males were used in statistical analysis to ensure equal male moth density among plots and to extend the time period during which the data could be collected.

Data analysis

Mating success of females was analyzed using the General Linear Model ANOVA procedure with Tukey's adjustment for multiple comparisons (Proc GLM; SAS Institute, 2008). The arcsin$\sqrt{x}$-transformed proportion of fertilized females was modeled as a function of week, dosage and block with interactions of factors. The interaction of dosage and block was used as error term.

Male moth catches in pheromone-baited traps were analyzed both by using data from the entire period of the study and separately by using data from various time intervals after pheromone application. We used Proc GLM ANOVA with Tukey's adjustment to test for significance of differences in moth counts between groups of traps located in plots treated with various doses and formulations of pheromone for each of the studies. To analyze pooled data, the $\ln(x + 1)$-transformed total moth counts per trap per week for each type of pheromone treatment was modeled as a function of week, dosage, and block with interactions of factors. The interaction of dosage and block was used as error term. For the analysis of trap catches during various time intervals, the $\ln(x + 1)$-transformed total moth counts per trap per week for each type of pheromone treatment was modeled as a function of dosage and block with interactions of

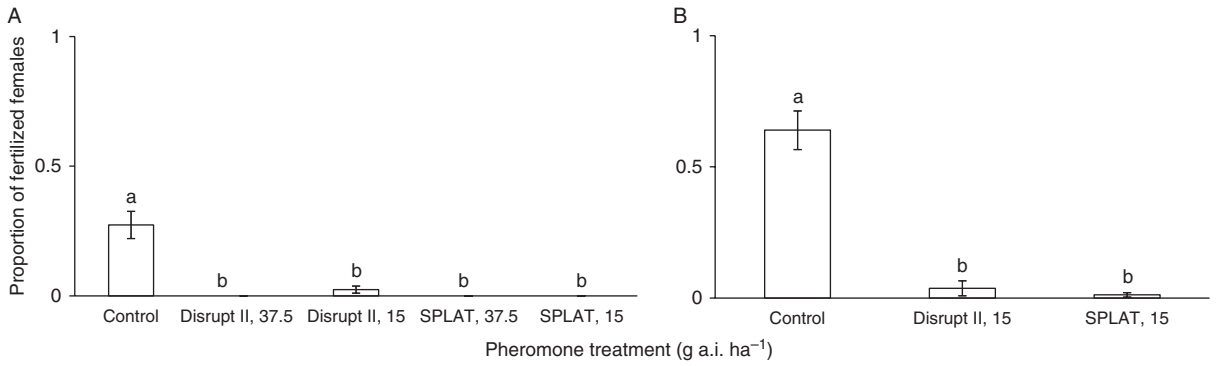

Figure 2 Mean (± SEM) proportion of fertilized females in plots treated with various dosages and formulations of pheromone in (A) 2006 and (B) 2007. Bars capped with the same letter are not significantly different (Tukey's HSD: P>0.05).

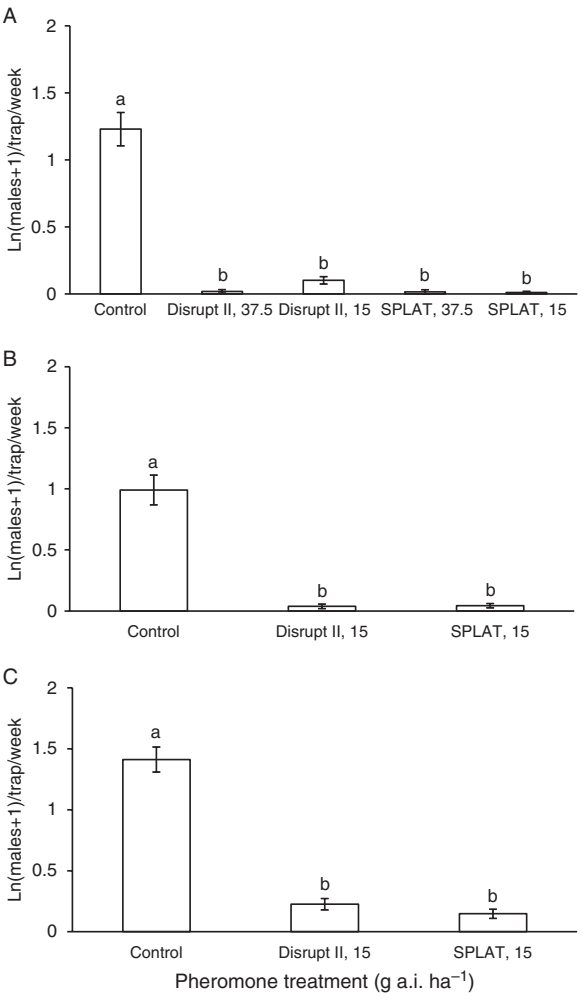

Figure 3 Mean (± SEM) weekly trap catch of gypsy moths [ln(no. males+1)] recaptured in plots treated with various formulations and dosages of pheromone in (A) 2006, (B) 2007, and (C) 2008. Bars capped with the same letter are not significantly different (Tukey's HSD: P>0.05).

factors. The interaction of dosage and block was used as error term.

Results

In 2006, female mating success was significantly reduced in the experimental plots compared with the control plots ($F_{4,3}$ = 13.5, P<0.001; Figure 2). Male moth catches in the pheromone-baited traps were also significantly reduced with all treatments ($F_{4,3}$ = 119.6, P<0.001; Figure 3). There was a significant effect of time ($F_{8,68}$ = 4.0, P<0.001) on male moth trap catch. However, the analysis of trap catch over time showed that during the first 5 weeks after its application, SPLAT® GM was as effective as Hercon Disrupt® II (Figure 4). Eleven weeks after the application, SPLAT® GM continued to reduce trap catches by >99% compared to control plots.

Similarly, in 2007, mating success of females was significantly reduced in the experimental plots compared with the control plots ($F_{2,3}$ = 83.8, P<0.001; Figure 2). Male moth trap catch was also significantly reduced in both treatments ($F_{2,2}$ = 121, P<0.001; Figure 3). The rest of the factors did not have a significant effect on male moth trap catch. No decrease in mating disruption was observed later in the season in the plots treated with SPLAT® GM (Figure 4).

In 2008, male moth trap catch was significantly lower in plots treated with dosages of 1.5 g a.i. ha^{-1} and higher, compared with the control plots ($F_{5,4}$ = 25, P<0.001; Figure 5). Dosages of 1.5 and 3 g a.i. ha^{-1} reduced trap catches by about 30%. SPLAT® GM was shown to be equally effective at 7.5 and 15 g a.i. ha^{-1}, reducing trap catches by over 90% compared to control plots. The two highest dosages of SPLAT® GM appeared to be as effective as Hercon Disrupt® II at 15 g a.i. ha^{-1}. Weekly analysis of male moth trap catch indicated that SPLAT® GM applied

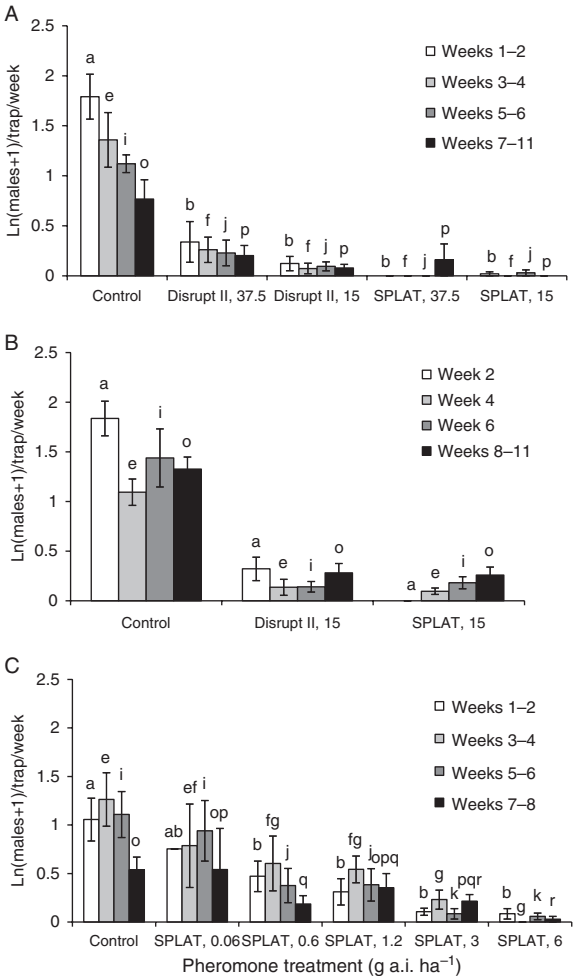

Figure 4 Mean (± SEM) weekly trap catch of gypsy moths [ln(no. males+1)] recaptured in plots treated with various dosages of pheromone formulated as Disrupt® II and SPLAT® GM in (A) 2006, (B) 2007, and (C) 2008. Within a series, bars capped with the same letters are not significantly different (Tukey's HSD: P>0.05). 2006: (a, b), (e, f), (i, j), and (o, p) indicate significant differences between trap catches at weeks 1–2, 3–4, 5–6, and 7–11, respectively. 2007: (a, b), (e, f), (i, j), and (o, p) indicate significant differences between trap catches at 2, 4, 6, and 8–11 weeks, respectively. 2008: (a–b), (e–g), (i–k), and (o–r) indicate significant differences between trap catches at weeks 1–2, 3–4, 5–6, and 7–8, respectively.

at 7.5 g a.i. ha^{-1} continues to be as effective as SPLAT® GM or Disrupt® II applied at 15 g a.i. ha^{-1} for the entire 8-week period.

Discussion

We sought to evaluate the effect of the new SPLAT® GM formulation on its ability to disrupt mating in gypsy moth

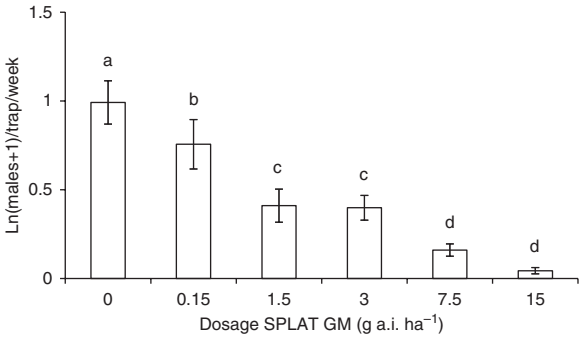

Figure 5 Mean (± SEM) weekly trap catch of gypsy moths [ln(no. males+1)] recaptured in plots treated with various dosages of pheromone in 2008. Bars capped with the same letter are not significantly different (Tukey's HSD: P>0.05).

populations and to compare its efficacy with that of Hercon Disrupt® II plastic flakes formulation, which is currently used for operational mating disruption treatments against gypsy moth. The results of all studies indicated that SPLAT® GM formulation was as effective as the Hercon Disrupt® II when applied at the same dosage and that it reduced mating success of females and male moth trap catch by >99% and >90%, respectively. The results of the dosage-response test also indicated that the effect of SPLAT® GM applied at 7.5 g a.i. ha^{-1} on male moth trap catch was comparable with both SPLAT® and Disrupt® II applied at the operational dosage of 15 g a.i. ha^{-1}.

To successfully disrupt mating in support of management programs, synthetic pheromones must be present in the air in sufficient quantities for the entire period of sexual activity of moths (Cardé et al., 1975; Howse et al., 1998). In the STS program, standard operating procedures require that the applied pheromone be effective for a period of at least 8 weeks to cover the entire period of gypsy moth flight, which generally occurs up to 6 weeks (Tobin et al., 2010), and to provide a safety margin for uncertainties associated with the logistics of treatment planning and gypsy moth phenology (Thorpe et al., 2006). The results of the analysis of weekly trap catch data indicated that SPLAT® GM applied at 7.5, 15, or 37.5 g a.i. ha^{-1} continues to be effective for at least 8 weeks and, therefore, would satisfy the above criterion for operational use in the STS program. Additional studies are needed to further investigate the effect of SPLAT® GM on gypsy moth mating success when applied at alternate dosages.

The gypsy moth continues to spread along a leading invasion front (Tobin et al., 2007). In addition, new populations are often detected in areas far from its current distribution, such as western North America (Ebata, 2009; Hajek & Tobin, 2009) and New Zealand (Glare, 2009). In many areas in which populations are detected, there are

concerns of non-target effects as well as environmental contamination. Mating disruption is the dominant tactic used against the gypsy moth in the STS program (Tobin & Blackburn, 2007), in part because it is both effective and more environmentally-friendly than alternatives. In eradication programs, such as those targeting incipient gypsy moth populations in western North America, the dominant tactic remains Btk (Hajek & Tobin, 2009) although there can be considerable public resistance to its use (e.g., East Bay Pesticide Alert, 2009). Consequently, mating disruption tactics could be an alternate control option in eradication efforts. Regardless of where mating disruption tactics are used, the development of an additional product will likely improve cost-effectiveness through competition among the formulators and applicators. Our field experiments demonstrated that the new formulation of disparlure, SPLAT® GM, significantly reduces mating success of deployed females and the number of male moths caught in deployed pheromone-baited traps when compared to control plots, and in a manner that is statistically the same as the current available mating disruption product, Disrupt® II.

Therefore, SPLAT® GM has been fully integrated into the STS program. In 2009, it has been aerially applied to ca. 41 000 ha. As a result of introduction of SPLAT® GM on the market the prices of treatments were lowered, which allowed STS program to treat an additional 5% ha with the same budget.

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