INTER-PLANT MOVEMENT AND SUPPRESSION OF TOBACCO BUDWORM IN MIXTURES OF TRANSGENIC *BT* AND NON-TRANSGENIC COTTON

J. L. Halcomb,[1] J. H. Benedict,[2]
J. C. Correa[2] and D. R. Ring[3]
Graduate Student, Professor, Technician, Extension Specialist, respectively
Texas A&M University, Department of Entomology
College Station, TX[1];
Texas A&M University Research and Extension Center,
Corpus Christi, TX[2]
Louisiana Cooperative Extension Service,
Baton Rouge, LA[3]

Abstract

Inter-plant movement of first-, third- and fifth-instar tobacco budworm, *Heliothis virescens*, (F.) were observed in field plots containing mixtures of transgenic (*Bt*) cotton plants, *Gossypium hirsutum*, expressing the δ-endotoxin from the bacterium, *Bacillus thuringiensis* Berliner spp. *kurstaki*, and non-transgenic cotton. The ratios of *Bt*:non-*Bt* plants used in the mixture stands were: (1) 100: 0, (2) 90:10, (3) 80:20, (4) 70:30, and (5) 0:100. We found that 37-38% of third- and fifth-instar tobacco budworms moved at least one plant in 24- and 48-hour periods. There was a trend for fifth-instar tobacco budworms to move greater distances and a greater number of plants as the proportion of *Bt* plants increased in the mixed stands compared with pure stands of non-*Bt* cotton. Fifth-instar tobacco budworms moved a significantly greater distance in the pure stand of *Bt* than in the pure stand of non-*Bt* at 24 hours after infestation. Third-instar tobacco budworms moved a shorter distance and less often than fifth-instars.

A field study was also conducted in 1994 and 1995 to determine the efficacy of different *Bt* and non-*Bt* plant mixtures on suppression of tobacco budworm and bollworm, *Helicoverpa zea* (Boddie). The mixture ratios were: (1) 100:0, (2) 90:10, (3) 85:15, (4) 80:20, (5) 75:25, and (6) 0:100. One other treatment (7) 0:100 was sprayed as needed for lepidopteran control. The other six treatments were not sprayed to suppress tobacco budworm or bollworm. As the proportion of non-*Bt* plants increased in a mixture, the number of larvae and amount of flower bud and boll injury increased for both years. Terminal damage also increased as *Bt* plants were decreased in mixtures in 1995.

Lint yield increased as the density of *Bt* plants in a mixture increased. The number of eggs in terminals was not significantly different between mixtures and pure stands of *Bt* or non-*Bt*. The number of injured flower buds, bolls, terminals and number of larvae in the pure stand of non-*Bt* that was sprayed for lepidopteran control was not significantly different from the pure stand of non-*Bt* that was not sprayed.

These data suggest that tobacco budworms and bollworms can develop on non-*Bt* plants in mixtures, move to *Bt* plants, feed, cause injury and be selected for resistance. Moreover, all adult tobacco budworms and boll-worms produced from mixed stands containing *Bt* cotton plants will have fed on *Bt* plants and have been selected for resistance to the *Bt* toxin.

Introduction

Transgenic (*Bt*) cotton expressing the δ-endotoxin from the bacterium, *Bacillus thuringiensis* Berliner spp *kurstaki*, has been effective in controlling the bollworm, *Helicoverpa zea* (Boddie) and tobacco budworm, *Heliothis virescens* (F.), in field trials (Benedict et al., 1991; Mahaffey et al., 1994; Benedict et al., 1996). A main concern however, is that with the expected widespread use of *Bt* cotton the tobacco budworm could adapt to the toxin thereby making the *Bt* cotton an ineffective tool for control. Gould et al. (1995) has demonstrated that the tobacco budworm is capable of developing high levels of physiological resistance to the δ-endotoxin.

Many strategies have been proposed for resistant management (Gould and Anderson, 1991; McGaughey and Whalon, 1992; Tabashnik, 1994). Before any strategy can be predicted to work best, knowledge of the inheritance of resistance and the behavior of tobacco budworm in relation to the *Bt* plant needs to be known. Some behavioral studies have indicated that third-instar tobacco budworms have increased spin-down and abandon plants more frequently on some *Bt* lines as compared to non-*Bt* lines (Benedict et al., 1993). One approach to manage resistance is the use of refuges to produce susceptible tobacco budworms. The use of mixed seed plantings has been theorized as one method to provide this refuge. It is suggested that manage-ment strategies will be influenced by movement of insects between the plants and the ability of the non-*Bt* plants to withstand injury (Tabashnik, 1994). Mixtures will work best if the movement of the tobacco budworm is limited between the *Bt* and the non-*Bt* plants (Mallet and Porter, 1992). However, the exact amount and percentage of movement within different mixtures of *Bt*:non-*Bt* stands has not been completely evaluated in the field. Preliminary studies in the greenhouse have indicated that 66% of third-instar tobacco budworm abandon or move at least one plant in 24-hours (Benedict et al., 1994). Parker and Luttrell (1995) have also shown inter-plant movement in mixtures.

The objectives of this study were: (1) to determine the amount of inter-plant movement of tobacco budworm in

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 2:924-927 (1996)
National Cotton Council, Memphis TN

mixed stands of *Bt* and non-*Bt* plants, (2) to determine the highest density of non-*Bt* cottonseed that can be mixed with *Bt* cottonseed that will maintain acceptable insect control properties, and (3) to assess the quality of mixed stands as refuges for tobacco budworms.

Materials and Methods

One inter-plant movement study was conducted in the field in 1995 at Texas Agricultural Experiment Station, Corpus Christi, Texas, and two insect control studies were conducted in 1994 and 1995 at Asgrow Research Farm, West Sinton, Texas.

Inter-Plant Movement on Mixtures of *Bt* and Non-*Bt* Cotton Plants. Two isolines were used: (1) a transformed line (*Bt*), expressing the *cryIA(c)* gene known as Delta Pine NuCOTN33B (2) a non-transformed line, from the same commercial variety, but without the *cryIA(c)* gene known as Delta Pine 5415. The seeds from the two isolines were premixed in the following proportions: *Bt* : non-*Bt* (1) 100: 0, (2) 90:10, (3) 80:20, (4) 70:30, (5) 0:100. Plants were grown in the field under normal agronomic practices.

Insects. The tobacco budworms used for this study were obtained from the USDA-ARS Southern Field Crops Insect Management Laboratory in Stone-ville, MS. This lab periodically mixes field-collected tobacco budworms to prevent the development of a “laboratory strain.” The eggs were held in an environmental chamber at 25±2°C until hatch. Upon hatching the neonates were placed individually on freshly picked non-*Bt* flower buds within 30 ml cups. They were held in an environmental chamber at 27± 2°C and 14:10 L:D cycle. The tobacco budworms were reared to the appropriate instar, third- or fifth-instar, for this study. Flower buds were replaced every two days.

Experimental Design. Six plant stands (mixtures) were planted in a randomized complete block design in plots consisting of a single 30-foot long row (38-inch between rows). Plants within each plot were bioassayed using tobacco budworm neonates on leaf disks to identify *Bt* and non-*Bt* plants. Two days prior to the release of larvae, all plots were over sprayed with malathion and pyronone to remove natural enemies. The study was conducted prior to natural infestation of tobacco budworm and bollworm, thus the only tobacco budworm larvae in the plots were those released in this study.

Three larvae of either third- or fifth-instar were placed on the top three leaves of a non-*Bt* cotton plant within each 30-foot row, except for the 100:0 mixture. Larvae were caged on the plants for four hours, then the cage was removed with as little disturbance to the plant as possible. Twenty-four and 48 hours after removal of the cage the following data was collected: (1) the number of plants moved, (2) the horizontal distance (cm) moved, (3) the number of larvae missing, (4) the number of larvae moved, and (5) the number of larvae dead. Neonates were placed on the terminal leaf of either *Bt* or non-*Bt* plants and observed for 2 hours.

Suppression of Tobacco Budworm in Mixtures

The suppression studies were conducted in the field at the Asgrow Research Farms in West Sinton, Texas during 1994 and 1995.

Plants. In 1994 and 1995 the two isolines used were (1) a transformed line expressing the *cryIA* gene, MON 531, and (2) a non-transformed line, 'Coker 312'. The seed from the two isolines were provided and pre-mixed in the desired ratios by Dr. Bob Buehler (Monsanto, St. Louis, MO). Plants were grown under normal agronomic practices.

Experimental Design

Cotton plants were planted in a randomized complete block design. The *Bt*:non-*Bt* ratios used in the mixtures were: (1) 100:0, (2) 90:10, (3) 85:15, (4) 80:20, (5) 75:25, (6) 0:100, and (7) 0:100 sprayed as needed for lepidopteran control. Plots consisted of four rows, 30 ft long (38 in between rows) with six replications. All treatments were sprayed for non-lepidopterous pests. Treatment (7) 0:100 was sprayed with Karate for tobacco budworm and bollworm control on 29 June and 6 July, 1994; and 7, 14, 21 and 28 July 1995. In 1994 data collected weekly for tobacco budworm and bollworm injury were number of injured flower buds and bolls and number of larvae in flower buds and bolls. Twenty flower buds and bolls were randomly sampled from the middle two rows of each plot for injury and number of larvae present per each sample date. In 1995 data collected weekly were number of eggs on terminals, number of injured flower buds, bolls, terminals and number of larvae in flower buds and bolls. Twenty flower buds, bolls and terminals were sampled in each plot per sample date. The species proportion of tobacco budworm and bollworm on non-*Bt* cotton was determined by sampling the border rows of each test (Table 5).

On 14 July 1994 during peak infestation the species composition and the density of fourth- and fifth-instar tobacco budworm and bollworm in the mixed stands were determined by counting the larvae in the middle two rows. On 24 and 25 July 1995 all instars of tobacco budworm and bollworm were collected from the outside two rows to determine the exact species composition and density.

Yields were determined by hand-harvested seedcotton from the middle two rows of each plot. The percent lint was determined by ginning seedcotton using a laboratory gin.

Statistical Analyses

Data from the inter-plant movement study was analyzed using analysis of variance and contrasts (PROC GLM, SAS

Institute 1988). The suppression study data was analyzed using analysis of variance and Fisher's protected LSD.

Results and Discussion

Inter-Plant Movement Study

Neonates did not move between plants in 24 hours, but remained on the plant they were originally placed on. The neonates crawled over the bottom and top of the terminal leaves for about 30 minutes prior to burrowing into the terminal of the plant. Observations were consistent with data by Parker (1995); however, increased locomotion was not noted on the *Bt* plants as compared to the non-*Bt* plants.

There was a trend for a greater percentage of inter-plant movement among fifth-instars compared with third-instars over all mixtures. Fewer third-instar tobacco budworms were recovered after a 24-hour period as compared to fifth-instars (Table 1). Approximately 38% of third- and fifth-instar tobacco budworms moved at least one plant in each 24-hour period. Significantly more fifth-instar tobacco budworms were not found in the 100% *Bt* plant stand (44.4%) as compared to the 100% non-*Bt* plant stand (16.7%) at the end of 24 hours. Also significantly more third-instars were not found (53.3%) in the pure *Bt* stand as compared to the 70:30 mixture (29.1%).

No statistically significant differences in the distances moved were found between the plant stands for third-instar tobacco budworms at 24 and 48 hours after infestation (Tables 2 & 3). Fifth-instars moved a significantly greater distance in the pure stand of *Bt* as compared to the pure stand of non-*Bt* and the 70:30 mixture at the end of 24 hours. There was a trend for the fifth-instars to move a greater distances, thus a greater number of plants, when the plant stands contained a higher proportion of *Bt* as compared to the pure stand of non-*Bt*. There was a trend for the fifth-instars to move a greater distance as compared to third-instars (Table 2).

Suppression Study

As the percentage of non-*Bt* plants increased in a plant stand, the number of fourth- and fifth-instar tobacco budworm and bollworm increased (Table 4). In 1994, the larvae were not collected therefore the exact species composition was not determined.

Differences could be seen in the season total number of injured flower buds, bolls and larvae for 1994 (Tables 6 and 7). There was a trend for increased flower bud, boll injury, terminal damage and number of larvae as the proportion of non-*Bt* plants increased in mixtures. All mixed stands with *Bt* had significantly less flower bud, boll and terminal damage than the pure non-*Bt* stand sprayed for bollworms and tobacco budworms. These trends were not always consistent, especially in the 75% and 80% mixtures. The number of eggs laid in terminals was not significantly different between any plant stand. Also, yields increased as the density of *Bt* plants increased in plant stands (Table 8). The yields of all mixed stands and pure *Bt* cotton plants also were greater than the pure stand of sprayed non-*Bt* in 1994. This was consistant with other experimental data on mixtures of *Bt* and non-*Bt* (Durant 1995). Significant differences between mixtures were not found in yield for 1995 although there was a trend for increased yield. All mixtures containing *Bt* cotton plants appeared to be efficacious in controlling the tobacco budworm and bollworm compared to pure stands of both sprayed and unsprayed non-*Bt* cotton.

These studies show that a large percentage of third- and fifth-instar tobacco budworms move plant-to-plant within plant stands composed of either 100% *Bt* and 100% non-*Bt* or mixtures. Late instars (fifth) can survive as demonstrated in the mixed field trials. Laboratory studies have shown that 48% of fifth-instar tobacco budworms and bollworms can feed on *Bt* cotton, pupate and become adults (Halcomb et al. 1994). On this basis we suggest that, in commercial production, mixed stands may produce late instar tobacco budworms and bollworms which could move to a *Bt* plant to feed, cause injury, be selected for resistance to the toxin, and develop into adults. Therefore, a mixed seed strategy will not provide a refuge that produces susceptible, non-selected adult moths.

Acknowledgment

We thank Ashley Rossi, Christie Day, Grace Moat, Tim Worden, Mollie Reese, Betsy Grubbs, and Troy Todd for assistance in data collection. A special thanks to Carolyn Villanueva for editing and word processing. We also thank Drs. Bob Buehler, Randy Deaton, Eric Johnson, and Laura Bradshaw for their assistance in conducting these studies.

References

Benedict, J. H., D W. Altman, E. S. Sachs, W. R. Deaton, and D. R. Ring. 1991. Field performance of cotton genetically-modified to express insecti-cidal protein from *Bacillus thuringiensis* III. College Station, p. 577. *In* J. M. Brown and D. A. Richter (eds.) Proc. Beltwide Cot. Prod. Res. Conf., National Cotton Council of America, Memphis, Tenn.

Benedict, J. H., E. S. Sachs, D. W. Altman, D. R. Ring, T. B. Stone and S. R. Sims. 1993. Impact of δ-endotoxin-producing transgenic cotton on insect-plant interactions with *Heliothis virescens* and *Helicoverpa zea* (Lepidoptera: Noctuidae). Environ. Entomol. 22: 1-9.

Benedict, J. H., E. S. Sachs, J. L. Halcomb, D. R. Ring, B. Cook, D. M. Stelly, D. W. Altman, R. J. Kohel, J. C. Correa, M. W. Goynes, J. F. Taylor and S. K. Davis. 1994. Feeding preference and movement of tobacco budworm and bollworm in mixed stands of transgenic *Bt* and non-*Bt* cotton. Annual Plant Resistance to Insects Newsletter. 20: 31-32.

Benedict, J. H., E. S. Sachs, D. W. Altman, W. R. Deaton, R. J. Kohel, D. R. Ring, and S. A. Berberich. 1996. Field performance of cottons expressing trangenic CryIA insecticidal proteins for resistance to *Heliothis virescens* and *Helicoverpa zea* (Lepidoptera: Noctuidae). J. Econ. Entomol. 89 (In Press).

Durant J. 1995. Efficacy of selected seed mixes of transgenic *Bt* and nontransgenic cotton against bollworms and tobacco budworms in South Carolina, pp. 769-771. *In* D. A. Richter and J. Armour (eds.). Proceedings Beltwide Cotton Conferences, National Cotton Council of America, Memphis, TN.

Gould, F. and A. Anderson. 1991. Effects of *Bacillus thuringiensis* and HD-73 delta endotoxin on growth, behavior, and fitness of susceptible and toxin-adapted strains of *Heliothis virescens* (Lepidoptera: Noctuidae). Environ. Entomol. 20: 30-38.

Gould, F. A. Anderson, A. Reynolds, L. Bumgarner and W. Moar. 1995. Selection and genetic analysis of a strain of *Heliothis virescens* (Lepidoptera: Noctuidae) strain with high levels of resistance to *Bacillus thuringiensis* toxins. J. Econ. Entomol. 88(6): 1545-1559.

Halcomb, J. L., J. H. Benedict, D. R. Ring and B. Cook 1994. Instar specific survival and feeding preference of bollworm and tobacco budworm in mixtures of transgenic *Bt* and nontransgenic cotton, pp. 1068-1072. *In* D. J. Herber and D. A. Richter (eds.). Proceedings Beltwide Cotton Conferences, National Cotton Council of America, Memphis, TN.

Mahaffey, J. S., J. S. Bacheler, J. R. Bradley, Jr and J. W. Van Duyn. 1994. Performance of Monsanto's transgenic *B. t.* cotton against high populations of lepidopterous pests in North Carolina, pp. 1061-1063. *In* D. J. Herber and D. A. Richter (eds.). Proceedings Beltwide Cotton Conferences, National Cotton Council of America, Memphis, TN.

McGaughey W. H. and M. E. Whalon. 1992. Managing insect resistance to *Bacillus thuringiensis* toxins. Science 258: 1451-1455.

Mallet, J. and P. Porter. 1992. Preventing insect adaptation to insect resistant crops: are seed mixtures or refugia the best strategy? Proc. R. Soc. London Ser B. 250:165-179.

Parker, C. D. Jr. and R. G. Luttrell. 1995. Inter-plant movement of tobacco budworm larvae in mixed plantings of *Bt* and non-*Bt* cotton., pp. 775-778. *In* D. A. Richter and J. Armour (eds.). Proceedings Beltwide Cotton Conferences, National Cotton Council of America, Memphis, TN.

SAS Institute. 1988. SAS/STAT user guide: statistics. SAS Institute. Cary, NC.

Tabashnik, B. 1994. Evolution of resistance to *Bacillus thuringiensis.* Annu. Rev. Entomol. 39:47-79.

Table 1. Inter-plant movement of third- and fifth-instar tobacco budworms after 24 and 48 hours in pure and mixed stands of *Bt* and non-*Bt* cotton plants in the field, 1995.

Mixture	24 hours		48 hours	
(*Bt*:non)	% moved	% missing	% moved	% missing
Third-instar				
100 :0	28.9 bc	53.3 a	11.9 c	73.8 a
90 : 10	30.9 bc	47.6 ab	13.3 bc	70.0 ab
80 : 20	29.6 bc	51.8 ab	50.0 ab	42.0 bc
70 : 30	41.6 bc	29.1 bc	77.7 a	22.2 bc
0 : 100	24.2 c	51.5 ab	36.3 bc	51.5 b
Fifth-instar				
100 : 0	38.9 bc	44.4 ab	61.1 ab	22.2 bc
90 : 10	66.6 ab	18.5 c	48.1 ab	51.8 b
80 : 20	80.0 a	13.3 c	73.2 a	13.3 c
70 : 30	55.5 ab	33.3 bc	50.0 ab	38.9 bc
0 : 100	55.5 ab	16.7 c	38.9 ab	33.3 bc

Means in a column followed by a different letter are significantly different (α=0.05) based on Contrasts (SAS Institute, 1988).

Table 2. Distance and number of plants moved by third- and fifth-instar tobacco budworms in pure and mixed stands of *Bt* and non-*Bt* cotton plants in the field at 24 hrs after infestation, 1995.

Mixture (*Bt*:non)	n	Distance (cm)	No. plants moved
Third-instar			
100 :0	21	20.2 c	1.8 b
90 : 10	24	20.3 c	1.7 b
80 : 20	13	33.6 abc	3.0 ab
70 : 30	17	13.6 c	1.2 b
0 : 100	16	20.1 c	1.9 b
Fifth-instar			
100 : 0	10	60.0 a	5.0 a
90 : 10	22	30.2 abc	2.5 ab
80 : 20	13	47.8 ab	4.7 a
70 : 30	12	29.0 bc	2.5 ab
0 : 100	15	22.3 bc	1.9 b

Means in a column followed by a different letter are significantly different (α=0.05) based on Contrasts (SAS Institute, 1988).

Table 3. Distance and number of plants moved by third- and fifth-instar tobacco budworm in pure and mixed stands of *Bt* and non-*Bt* cotton plants in the field at 48 hrs after infestation, 1995.

Mixture (*Bt*:non)	n	Distance (cm)	No. plants moved
Third-instar			
100 :0	10	23.6 ab	2.5 abc
90 : 10	9	12.4 b	1.2 c
80 : 20	7	42.1 ab	3.6 abc
70 : 30	7	33.6 ab	3.3 abc
0 : 100	15	35.8 ab	2.5 bc
Fifth-instar			
100 : 0	15	58.6 a	4.3 ab
90 : 10	13	42.3 ab	3.6 abc
80 : 20	12	63.0 a	5.8 a
70 : 30	11	42.7 ab	4.2 abc
0 : 100	12	35.3 ab	2.9 abc

Means in a column followed by a different letter are significantly different (α=0.05) based on Contrasts (SAS Institute, 1985).

Table 4. Number of fourth- and fifth-instar tobacco budworm and bollworm larvae in pure and mixed stands of *Bt* and non-*Bt* cotton plants in the field, 1994 and 1995.

	1995		1994
Mixture	Tobacco budworm	Bollworm	Tobacco budworm/bollworm
100 : 0	0.0 b	0.3 a	3.8 d
90 : 10	0.2 b	0.5 a	6.2 cd
85 : 15	0.3 b	0.2 a	7.2 bcd
80 : 20	1.0 ab	0.2 a	10.5 b
75 : 25	0.8 ab	0.5 a	9.0 bc
0 : 100	1.2 ab	0.0 a	20.2 a
0 : 100 Spray @ 10%	2.0 a	0.0 a	—*

Means followed by different letters are significantly different (α = 0.05) Fisher's protected LSD. In 1994 the exact number of each species was not determined.
* No data was collected.

Table 5. Proportion of bollworm and tobacco budworm in the natural larval population on non-*Bt* cotton, unsprayed for Lepidoptera, 1994 and 1995.

Date	n	% bollworm	% tobacco budworm
6/28/94	27	89	11
7/5/94	31	35	65
7/12/94	25	32	68
7/7/95	50	64	36
7/24/95	26	4	96

Table 6. Season total number of injured flower buds, bolls and number of larvae found in squares and bolls in pure and mixed stands of *Bt* and non-*Bt* cotton plants in the field, 1994.

Mixture	No. injured flower buds	No. injured bolls	No. larvae in squares & bolls
100 : 0	0.5 b	0.7 b	0.2 b
90 : 10	1.3 b	2.5 b	1.3 cd
85 : 15	2.5 b	3.5 b	1.7 cd
80 : 20	2.0 b	1.8 b	1.2 cd
75 : 25	2.8 b	2.5 b	3.0 c
0 : 100	13.0 a	10.0 a	10.3 a
0 : 100 Spray @ 10%	11.2 a	8.5 a	6.5 b

Means followed by different letters are significantly different (α = 0.05), Fisher's protected LSD.

Table 7. Season total number of bollworm/budworm eggs in terminals, damaged terminals, injured squares and bolls, and larvae found in squares and bolls in pure and mixed stands of *Bt* and non-*Bt* cotton plants in the field, 1995.

Mixture	No. eggs in terminals	No. damaged terminals	No. injured squares	No. injured bolls	No. larvae in squares & bolls
100 : 0	11.3 ab	4.2 c	0.5 d	0.8 b	0.2 b
90 : 10	8.7 b	5.2 bc	1.7 cd	1.5 b	0.5 b
85 : 15	15.0 a	8.0 bc	1.2 cd	2.0 b	0.8 b
80 : 20	8.5 b	9.0 b	3.0 bc	2.0 b	1.3 ab
75 : 25	8.8 b	8.2 bc	2.8 bc	2.3 b	0.8 b
0 : 100	9.5 ab	17.7 a	4.8 ab	4.8 a	2.7 a
0 : 100 Spray @ 10%	11.7 ab	17.3 a	5.5 a	2.8 ab	2.3 a

Means followed by different letters are significantly different (α = 0.05), Fisher's protected LSD.

Table 8. Lint yield in pure and mixed stands of *Bt* and non-*Bt* cotton plant in the field, 1994 and 1995.

Mixture	1994	1995
100:0	1154 a	648 ab
90:10	1048 ab	665 a
85:15	1017 ab	655 ab
80:20	975 b	648 ab
75:25	968 b	648 ab
0:100	443 d	615 ab
0:100 Spray @ 10%	714 c	631 ab

Means followed by different letters are significantly different (α = 0.05), Fisher's protected LSD.