# Biology and Mortality Agents of the Fruittree Leafroller (Lepidoptera: Tortricidae), on Baldcypress in Louisiana<sup>1</sup>

David M. Braun, Richard A. Goyer, and Gerald J. Lenhard<sup>2</sup>

Department of Entomology, Louisiana Agricultural Experiment Station Louisiana State University Agricultural Center Baton Rouge, LA 70803

J. Entomol. Sci. 25(1): 176-184 (January 1990)

**ABSTRACT** The fruittree leafroller, Archips argyrospila (Walker), recently has been found feeding on a new host, baldcypress (*Taxodium distichum* (L.) Rich.) at outbreak levels in Louisiana. The general biology of *A. argyrospila* and the impact of its parasitoids, predators, and hyperparasitoids found in infestation areas in and near the Atchafalaya Basin in Louisiana are described. The hymenopteran parasitoid, *Itoplectis conquisitor* (Say), caused the greatest amount of pupal parasitism. Two carabids, *Plochionus timidis* Haldeman and *Calleida viridipennis* Say, were found to be important predators of larvae and pupae.

**KEY WORDS** Parasitoids, predators, Archips argyrospila, baldcypress, leafroller, Carabidae, Tortricidae, Taxodium distichum, Plochionus timidis, Calleida viridipennis, Louisiana.

The fruittree leafroller (FTL), Archips argyrospila (Walker) (Lepidoptera: Tortricidae), has been found feeding on baldcypress (*Taxodium distichum* (L.) Rich.) since 1983 in 6 southern Louisiana parishes. This was the first report of this insect on baldcypress. Aerial surveys conducted annually by the authors have found as many as 200,000 acres of baldcypress forest affected by this insect in southern Louisiana (Goyer and Lenhard, 1988).

Defoliation in cypress stands often is patchy, typically concentrated on edge trees 20 to 45 cm diameter above butt swell, or the lower crown of larger trees along the edge of natural or man-made waterways. The fruittree leafroller (FTL) has been recognized as a major pest of apple (*Malus domestica* (Borkh)) throughout the United States since 1913 (Chapman and Lienk, 1971; Gill, 1913). Other principal hosts in the United States also include oaks (*Quercus* spp.) and hawthorn (*Crataegus* spp.). Other trees and shrubs are eaten only during outbreak conditions (Chapman and Lienk, 1971).

Due to the potentially devastating effects this defoliator could have on both growth loss and tree mortality, we needed to know the major parasitoids and predators of the FTL, and determine the capacity of these beneficials to regulate population density. Data were collected in 1984, 1985, 1987, and 1988. The overall objective of the research is to develop techniques to provide land managers with accurate predictions of the intensity and impact of *A. argyrospila* outbreaks in

<sup>&</sup>lt;sup>1</sup> Accepted for publication 31 October 1989.

<sup>&</sup>lt;sup>2</sup> Former Research Associate, Professor, and Research Associate, respectively.

baldcypress stands. This portion of our study was directed at comparing the biology of this tortricid with that reported earlier on orchard trees, and evaluating the impact of natural enemies on survivorship.

## **Materials and Methods**

Field populations of the FTL have been monitored throughout its known Louisiana range since its first reported occurrence on baldcypress in 1983. In 1984, 1985, 1987, and 1988, FTL pupae were field collected by hand and pole pruner from lower and mid-crown levels of baldcypress and were reared in the laboratory for larval-pupal and pupal parasitoids each year from 1984 - 1988 (excluding 1986). Additionally, FTL egg masses were field collected monthly, June - February, and reared in the laboratory for egg parasitoids. During 1987, FTL pupae were collected from foliage, as well as from under burlap bands at two plots which had shown different population levels the previous year. Unpaired t-tests (Steel and Torrie, 1980) were used to compare plots and collection methods.

During the years of 1985 and 1988, infested baldcypress foliage was intensively evaluated to estimate predation by the two frequently occurring species of carabid beetles, *Calleida viridipennis* Say and *Plochionus timidus* Haldeman, found to be associated with the FTL. Infested foliage was selected from the mid to lower crown and webbed leaves were examined. Counts were tallied as empty, there was no evidence of a dead or alive larva; live, a healthy appearing larva; or dead, larva exhibited evidence of having been fed upon by carabids. Laboratory rearings have demonstrated a characteristic blackened and shrunken appearance of FTL larvae after carabid feeding.

During the spring of 1987, two study areas were selected to compare the impact of parasitoids and predators in a low and high density FTL population and to ascertain the stages of FTL development at which mortality occurred. One area, "Frank's Lake," in Iberville Parish, had been observed to have a lower egg mass population of FTL than the second area, "Eel Tree," in lower Saint Martin Parish. Both areas contained seasonally flooded, second-growth baldcypress forest. Bald-cypress was the predominant tree species, with water tupelo (Nyssa aquatica L.), green ash (Fraxinus pennsylvanica (Borkh.) Sarg.), black willow (Salix nigra Marsh), and red maple (Acer rubrum L.) as dominant, less abundant, associates. Buttonbush (Cephalanthus occidentalis L.) was the major component of the few understory shrubs present in this semi-aquatic ecosystem.

Study trees at Frank's Lake were in a shallow lake bordering and serving as a seasonal overflow for Grand River, while those at Eel Tree bordered a distributary of Grand River, ca. 35 km downstream. Study trees were 20 to 35 cm diameter above butt swell, and 15 to 30 m in height, growing on or near the edge of open water and most possessed full or nearly full crowns.

During 1987, two passive collection methods were used to sample crawling and flying FTL natural enemies. Burlap bands 10 cm wide were tied to trunks of baldcypress, black willow, water tupelo, and red maple study trees approx. 2-3 m above the water level. Other trees had Tanglefoot<sup>®</sup> applied in bands 10 cm wide directly onto the bark. Burlap bands and sticky traps were checked ca. weekly, and all insects were removed and identified. Six baldcypress trees were chosen at each site for in-depth sampling and are referred to as intensive study trees in this paper. Each of the 12 trees had 30 cm wide burlap bands tied with string at head height, and were folded to provide a secluded habitat to concentrate crawling insects.

Additionally, foliage samples were taken from each tree at mid and upper crown levels ca. weekly. On each date, 20-30 FTL larvae were recovered from each tree for instar determination via head capsule measurement. The ranges in head capsule width that represented each of the 5 instars of the FTL on baldcypress had been determined from earlier data collected in the general locale of the study areas (Goyer, unpublished).

Exclusion bags were made of fine screen netting (80 mesh) supported by wire hoops. The bags covered 0.5 to 1.0 meter of branch tip, and were fastened with wire around a naturally infested baldcypress branch padded with non-absorbent cotton to act as a barrier to insect movement. This design facilitated removal and reinstallation of bags necessary during periodic checks. Bags were installed on a temporally staggered timetable at Eel Tree and Frank's Lake during the 1987 larval development period (Table 1) to permit pinpointing periods of parasitization.

Sixty-nine exclusion bags were distributed among the 6 intensive study trees at each site, located throughout the crowns of each. In order to minimize disturbance of the larvae, insects were not removed from their feeding webs when counted initially. Most larvae could be observed by inspecting the webs; those that could not were presumed to be in webs tightly bound with silk that resisted light finger pressure. Unoccupied webs displayed obvious characteristics: they flattened easily, and often were partially open. The number of larvae in the bags installed in the first installation at Frank's Lake, and the two at Eel Tree (Table 1), were later corrected to include larvae found in some of the bags in addition to those recorded in the original count. The ratio of larvae to leaves in each bag was ca. 1:10. If the number of larvae exceeded this ratio, webs were removed from the foliage at random until the approximate ratio was reached. Earlier observations had concluded that densities in excess of this ratio led to starvation (Goyer, unpublished). A few bags at each site had all their leaves eaten before the larvae reached pupation, and had to be moved to a nearby branch possessing some remaining leaves to prevent premature pupation or starvation. The proportions of different instars present in each bag at the time of bag placement were estimated from the population of FTL by extensive sampling of each tree within four days of bag installation. Each branch to be bagged was inspected for other insects and arachnids, and all those found were removed. Archips pupae were removed as they appeared, and were reared in the lab for ease of recovery of parasitoids and hyperparasitoids. Adults of any larval parasitoids, found either dead or alive during weekly bag checks, also were removed.

### **Results and Discussion**

On baldcypress in Louisiana, development and feeding behavior of the FTL were similar to what has been described on apple, but with some important exceptions. As described by Chapman and Lienk (1971), and Gill (1913), on apple, the fruittree leafroller is univoltine, with 5 instars. The development from egg hatch, which occurs concurrently with spring bud break, to adult emergence takes ca. 6 weeks in the north and east. In the outbreak areas in Louisiana, FTL development on baldcypress also was univoltine with 5 instars. However the total development time for FTL was considerably longer; we observed egg hatch in late

Table 1.	Table 1. Mortality of A.	of A. argyrospila life stages from exclusion bag study. 1987.	stages from	exclusion ba	ıg study. 19	87.		
			Total No.	Larval		Pupal		
	Life	No. of	of	Parasitism	Unknown	Parasitism	Unknown	
Date Bags	gs Stage	Exclusion	Larvae at	by	Larval	by $I$ .	Pupal	Total
Installed	Present*	Bags	Start	Braconidae	Mortality	conquisitor	Mortality †	Mortality
				(u) %	(u) %	% (n)	(u) %	(u) %
			TUT	TOLY JURE FLOI				
Apr 2	3	10	144	2.8 (4)	46 (66)	3 (2)	19 (14)	
Apr 17	3 & 4	12	151	2.6 (4)	48 (72)		36(27)	70 (105)
Apr 30	5 & P	11	91	0) 0	28 (25)	2 (1)	18 (12)	42 (91)
			FRANK	FRANK'S LAKE PLOT				
Apr 17	3 & 4	24	289	1 (3)	24 (68)	6 (13)	18 (40)	43 (124)
May 4	5 & P	12	115	(0) 0	27 (31)	1 (1)	46 (53)	74 (85)
* Indícates	instar of larva (3, 4, c	or 5th) or pupa (P).						
† Calculated	+ Calculated by the formula: $[# c$	- Calculated by the formula: $ #$ of pupae which died/(total no. of A. argyrospila at start all larval mortality) × 100.	otal no. of A. argy	<i>rospila</i> at start-al	ll larval mortality	y)] × 100.		

BRAUN, GOYER, and LENHARD: Biology of Fruittree Leafroller 179

February, with the final imagos emerging in late May to early June. The approximate doubling of development time may be due, in part, to the variability of bud break and irregular temperatures that occur in LA in February and March. Although FTL egg hatch appeared fairly well synchronized with leaf flush on individual trees, there was great variability between neighboring trees. Furthermore, there was variability, both within and among tree crowns in leaf morphology. (Further research is currently underway to determine if these variuos leaf morphs affect FTL development times). Also, because of wind-aided ballooning and subsequent dispersal to surrounding trees, two and occasionally three larval instars were commonly found together in foliage sampled from a single tree on any sample date.

On apple, egg masses are deposited on thin twigs; this is also true for baldcypress. On apple, larvae eat opening flower buds, newly set fruit, and leaves; but on baldcypress, the opening leaf buds and elongating leaves are eaten, while the monoecious flowers and developing conelets generally are not. Larval webs first cover opening buds, then roll together leaflets and, later, several whole baldcypress leaves. Larvae remain inconspicuous, to the passerby, on baldcypress unless the tree is severely defoliated, at which time they become very active, crawling on branches and spinning down on silk. This dispersal activity in search of foliage may increase their mortality rates, as they become more accessible to a great variety of predators. Often larvae fall into the standing water and drown or become prey for fish. Massive mortality to mature larvae was observed on many occasions where baldcypress were completely defoliated.

The predators, parasitoids, and hyperparasitoids of FTL collected in the study are listed in Table 2. The ichneumonid pupal parasitoid, Itoplectis conquisitor (Say), was recovered far more frequently than the chalcid, Brachymeria ovata (Say), from pupae collected from baldcypress (Table 3). The parasitism rates of L. conquisitor at Frank's Lake were not statistically different from that at Eel Tree (p>.05). Furthermore, there was no significant difference in rates of parasitism between FTL pupae collected from foliage compared to that of burlap band collections (p>.05). Brachymeria ovata was reared most frequently from pupae collected on foliage, with few from pupae collected under burlap. An infrequent parasitoid, Cirrospilus sp. near flavicinctus Riley, was reared from pupae collected in 1984-1985. The rate of parasitism by this eulophid was low and is included as "other parasitoids" in Table 4. Two species of hyperparasitoids were reared in approximately equal numbers from field-collected pupae in all years of this study. the pteromalid Cyclogastrella n. sp. has been reported earlier from Florida on Anacamptodes, an occasional pest of baldcypress. The eulophid Dimmockia incongrua (Ashmead) is a known hyperparasitoid in the eastern and southern U.S. (E.E. Grissel and M. E. Schauff, personal communication). Dissection of pupae from which the hyperparasitoids emerged showed prior parasitism by I. conquisitor. The larval parasitoid Apanteles polychrosidis Viereck was recovered infrequently from exclusion bags installed in early and mid-April at Eel Tree, and in mid-April at Frank's Lake. No parasitoids were recovered from the bags installed at Eel Tree on April 30, or from those installed in early May at Frank's Lake (Table 1).

Collections of FTL pupae during the years of 1984, 1985, 1987, and 1988 showed a range of parasitism from *I. conquisitor* of 8 to 37%, with an overall weighted averge of 19%. During the same period, *B. ovata* showed a parasitism rate of from 2 to 13%, averaging only 4% (Table 4). There appeared to be no

Family/Species	FTL Life Stage Attacked
INSECT PREDATORS	
REDUVIIDAE	
Arilus cristatus (L.)	Larva
PENTATOMIDAE	
Podisus maculiventris (Say)	Larva
CARABIDAE	
Plochionus timidus Haldeman	Larva, Pupa
Calleida viridipennis (Say)	Larva, Pupa
FORMICIDAE	
Camponotus rasilis (Wheeler)	Larva
VESPIDAE	
Polistes annularis (L.)	Larva, Pupa
P. exclamans Viereck	Larva, Pupa
LIBELLULIDAE	
Erythemis simplicicollis (Say)	Larva
VERTEBRATE PREDATORS	
IGUANIDAE	
Anolis carolinensis Voigt	Pupa
PARIDAE	
Parus carolinensis (Audubon)	Larva
PARULIDAE	
Protonotaria citrea (Bodaert)	Larva
INSECT PARASITOIDS	
ICHNEUMONIDAE	
Itoplectis conquisitor (Say)	Pupa
CHALCIDAE	
Brachymeria ovata (Say)	Pupa
BRACONIDAE	
Apanteles polychrosidis Viereck	Larva
EULOPHIDAE	
Cirrospilus sp. nr flavicinctus Riley	Pupa
INSECT HYPERPARASITOIDS	
PTEROMALIDAE	
Cyclogastrella n. sp.	Pupa ex. I. conquisitor
EULOPHIDAE	
Dimmockia incongrua (Ashmead)	Pupa ex. I. conquisitor

# Table 2. Observed predators, parasitoids, and hyperparasitoids of *A. argyrospila* (FTL) on baldcypress in Louisiana (1987).

obvious correlation between % parasitism and overall FTL population density. We did, however, encounter *B. ovata* more frequently in 1984 and 1985 while *I. conquisitor* was the dominant parasite species in 1987 and 1988.

Both vertebrate and invertebrate predators of FTL larvae and pupae were observed at the study areas (Table 2). Two carabid beetles, *C. viridipennis* and *P. timidus* appeared to be the most important invertebrate predators. Adults of both species were collected at Eel Tree in the first week of April and at Frank's Lake in

		FROM V	JNDE	R BURL	AP			
								E WITH
COLLECTION	STUDY*	A. argyrospila		nquisitor		ovata		RASITOIDS
DATE	AREA	PUPAE, n		% (n)	%	(n)	%	(n)
Apr 27	$\mathbf{ET}$	68	0	(0)	0	(0)	1.5	(1)
May 8	$\mathbf{ET}$	614	2	(13)	0	(0)	.0	(0)
subtotal	ET	682	0	(13)	0	(0)	0.2	(1)
Apr 27	$\mathbf{FL}$	114	4	(4)	0	(0)	6.0	(7)
May 4	$\mathbf{FL}$	205	22	(45)	0	(0)	.0	(0)
May 6	$\mathbf{FL}$	113	18	(20)	0	(0)	.0	(0)
subtotal	$\mathbf{FL}$	432	16	(69)	0	(0)	1.6	(7)
TOTAL	ET & FL	1114	8	(82)	0	(0)	0.7	(8)
		FRO	OM FC	<b>LIAGE</b>				
May 8	$\mathbf{ET}$	133	15	(20)	0	(0)	4.3	(1)
<b>May</b> 13	$\mathbf{ET}$	196	18	(35)	2	(3)	2.0	(4)
subtotal	ET	329	17	(55)	1	(3)	1.5	(5)
May 4	FL	23	0	(0)	0	(0)	4.3	(1)
May 12	$\mathbf{FL}$	85	20	(17)	0	(0)	2.0	(4)
May 18	$\mathbf{FL}$	331	14	(47)	5	(16)	1.5	(5)
May 27	$\mathbf{FL}$	16	0	(0)	0	(1)	37.5	(6)
subtotal	FL	455	14	(64)	4	(17)	2.6	(12)
TOTAL	ET & FL	784	15	(119)	3	(20)	2.2	(17)

Table 3.	Parasitism of A.	argyrospila	collected f	from b	oaldcypress i	in Louisiana,
	1987.					

\* ET = Eel Tree Plot, FL = Frank's Lake Plot

Table 4. Emergence of A. argyrospila and parasitoids from field-collectedpupae.

	1	1984	1	1985	1987		1988		TOTAL	
	%	(n)	%	(n)	%	(n)	%	(n)	%	(n)
A. argyrospila	64	(98)	76	(126)	83	(784)	61	(196)	76	(1204)
I conquistitor	22	(24)	8	(13)	14	(136)	37	(119)	19	(302)
B. ovata	13	(19)	12	(20)	2	(20)	2	(8)	4	(67)
Other Parasitoids	1	(1)	4	(7)	0	(0)	0	(0)	0	(8)
Total Parasitoids Total Insects	36	(54) (152)	24	(40) (166)	17	(156) (940)	39	(127) (323)	24	(377) (1581)

the third and fourth weeks of April. By the fourth week in April, larvae of both predatory species were observed in baldcypress foliage, feeding along with adult on FTL larvae in webs at both sites. Later, when some trees became completely defoliated, larvae of both predator and prey could be seen actively moving along bare branches and trunks, and their frequent predation was observed. Final instar larvae of the predators congregated under burlap bands, along with FTL larvae, presumably, to pupate. The highest concentration of predator and prey larvae found under a single burlap band was observed at Eel Tree where ca. 200 carabid larvae, both species combined, and 400 FTL larvae were found (May 8). Four carabid pupae also were found under the same band; this being the earliest date that predator pupae were found at either study site. FTL pupae had been found for the first time at the study site two weeks earlier. During 1985, 1212 FTL larvae were examined *in situ* for predation rates at four locations and an additional 560 in 1988 at three widely separated locations. Carabid predation ranged from 6 to 32% in 1985, averaging 18%, while rates ranged from 4 to 27% in 1988 with a mean of 15% (Table 5). No significant predation by these carabids was observed prior to 1985. It is unclear whether these two species are long-time or recent associates of FTL.

	198	35	198	8	TOTAL	
	Percent	(n)	Percent	(n)	Percent	(n)
HEALTHY	82	(995)	85	(474)	83	(1469)
ATTACKED	18	(217)	15	(86)	17	(303)
TOTAL		(1212)		(560)		(1772)

Table 5. Predation of Archips argyrospila on baldcypress in Louisiana.

Other insects observed feeding on FTL larvae and pupae are listed in Table 2. Importantly, *Polistes annularis* (L.) and *P. exclamans* Viereck nests occupied ca. 50% of the baldcypress in the study area and were observed frequently removing FTL larvae from webs. *Erythemis simplicicallis* (Say) captured FTL larvae as they were lowering themselves on silk especially in high density areas. Although never actually observed feeding in the field, two polyphagous hemipterans (*Podisus maculiventris* (Say) and *Arilus cristatus* (L.)) also were found commonly in baldcypress crowns. Both of these species readily accepted FTL larvae in the laboratory.

Vertebrate predators observed feeding on the FTL were the green anole lizard, Anolis carolinensis Voigt; and two bird species, the Carolina chickadee, Parus carolinensis (Audubon), and the prothonotary warbler, Protonotaria citrea (Bodaert). All three are common residents of the balcypress - tupelo ecosystem.

Although the two study sites were chosen initially because one area appeared to have the potential for a low level of infestation, and the other had an epidemic level in the previous year, both sites exhibited patterns of defoliation that were quite similar by late May of 1987. This occurred even though the parasitism rate of pupae collected at Frank's Lake was numerically greater than that at Eel Tree (Table 3).

Paradis and LeRoux (1965) concluded that declines of FTL outbreaks in Quebec were due to density-dependent bird populations and the parasitoid I. conquisitor. Chapman et al. (1941) found than an egg parasite, *Trichogrammatomia* tortricis (Girault) destroyed up to 89.4% of the FTL eggs. Chapman and Lienk (1971) believed that all three may be mortality agents that act as important factors in causing the decline of outbreaks on apple in New York state. Although I. conquisitor was found to be the most abundant pupal parasitoid in the outbreaks studied in Louisiana, it has apparently not had a great enough impact on the level of FTL to cause its decline to low population levels alone, or with the additional impact of the two species of insectivorous birds. The two carabid species found feeding on FTL larvae and pupae in Louisiana are occasionally abundant; however, they have not been implicated as important predators of FTL (or any other lepidopteran) during outbreaks elsewhere. Egg parasitoids have not been found in FTL in Louisiana. Several hundred egg masses collected at monthly intervals from 1984 to 1988 and reared in the laboratory did not yield a single egg parasite. This is one apparently important void in the natural control of FTL in Louisiana, and may warrant further study.

Although from their first discovery in 1983 local FTL densities have been observed to vary from year to year, area-wide decline in the population has not yet been seen. Therefore, it can be concluded that the mortality agents discussed in this paper are, at most, having a dampening effect on FTL population growth at the present time. The two intensive study areas exhibited pupal parasitism rates that differed, numerically, but, due to local variation, did not differ statistically. Itoplectis conquisitor was most abundant at both study sites, while B. ovata was reared most frequently from pupae collected from foliage in 1984 and 1985. Larval parasitism, as determined from the exclusion study, occurred at a very low rate, affecting only a few early-instar larvae. Egg parasitism was non-existent. Hyperparasitoids, from 8 to 20 or more per pupae, were found in pupae first parasitized by I. conquisitor. The hyperparasitoids affected a low proportion, however, and did not appear to significantly impact the abundance of *I. conquisitor*. The two species of carabids, and, possibly, native paper wasps, appeared to be the most important predators of larvae. Thus, further studies of the various mortality agents are needed before we can predict population trends for A. argyrospila affecting baldcypress in Louisiana.

### Acknowledgments

We wish to thank personnel of the Williams Company, Inc. of Patterson and New Orleans, LA for use of land and facilities at Indigo Island. The identification of insects were made by Drs. M. E. Schauff (Eulophidae), P. M. Marsh (Braconidae) and E. E. Grissell (Chalcidae and Pteromalidae) USDA, Beltsville, MD; Dr. T. L. Erwin (Carabidae), the Smithsonian Institution; and Dr. J. B. Chapin and C. Barr, Department of Entomology, LSU, for various predatory species. Thanks also to Drs. David Boethel and E. A. Heinrichs of this Department for constructive suggestions on an early version of this manuscript. Approved for publication by the Director of the Louisiana Agricultural Experiment Station as manuscript number 89-17-3043.

#### **References** Cited

- Chapman, P. J. and S. E. Lienk. 1971. Tortricid fauna of apple in New York (Lepidoptera: Tortricidae). N.Y. State Agric. Exp. Stn., Geneva. Spec. Publ. March, pp. 63-120.
- Chapman, P. J., G. W. Pearce and A. W. Avens. 1941. The use of petroleum oils as insecticides. III. Oil deposit and the control of fruit tree roller and other orchard pests. J. Econ. Entomol. 34: 639-647.
- Gill, J. B. 1913. Papers on deciduous fruit roller (Archips argyrospilus Walk.). U. S. Dept. Agric. Bur. Entomol. Bull. 116 (Part 5): 91-110.
- Goyer, R. A., and G. J. Lenhard. 1988. A new insect pest threatens baldcypress La. Agric. 31(4): 16-17, 21.
- Paradis, R. and E. J. LeRoux. 1965. Recherches sur la biologie et la dynamique des populations naturalles d'Archips argyrospilus (Lepidoptera: Tortricidae) dans le sud ouest de Quebec. Can. Entomol. Soc. Mem. No. 43. 77 pp.
- Steel, R. G. D. and J. H. Torrie. 1980. Principles and Procedures of Statistics. A Biometrical Approach. McGraw Hill, N.Y. 2nd Ed., 633 pp.