1984

Estimating Infestation Rates of the Nantucket Pine Tip Moth (Lepidoptera: Tortricidae) Through Sequential Sampling

David Kulhavy
Arthur Temple College of Forestry and Agriculture, Stephen F. Austin State University, dkulhavy@sfasu.edu

Paul C. Johnson
Arthur Temple College of Forestry and Agriculture, Stephen F. Austin State University

Bernard Andersen

Table 5. Regression analyses results for combined $\hat{P}_a$ and $\hat{P}_A$ for sequential sampling and systematic sampling for each site and generation for sites and sites combined

Site generation			r^2	n
I. OW[a] Systematic	0.15	0.41	0.91	92
OW Sequential	0.10	0.81	0.90	50
I Sequential	0.10	0.89	0.99	24
II Sequential	0.99	0.71	0.99	46
III Sequential	0.35	0.56	0.96	26
IV Sequential	0.07	0.47	0.94	21
Combined	0.57	0.49	0.85	259
II. I Systematic	0.12	0.62	0.94	77
I Sequential	0.62	0.32	0.98	40
II Sequential	0.36	0.80	0.97	36
III Sequential	−4.96	0.73	0.73	21
IV Sequential	0.33	0.70	0.91	35
Combined	0.31	0.71	0.87	209
Site combined	0.84	0.50	0.82	468

[a] OW, Overwintering generation.

quential sampling can be used as a rapid estimate of infested tips for economic damage and population trends, but would probably benefit from a stratified sampling approach where random samples are drawn from the perimeter and actual areas of the stand in proportion to their relative area.

No strong correlation could be found between top level and whole-tree infestations. This was true for all trees combined, trees <60 cm in height, and trees >60 cm in height. On Site II there was a highly significant correlation for all trees >60 cm, with $r^2 = 0.76$.

For sequential sampling to be practical for estimating NPTM infestation rates, the amount of variation between apparent infestations within the same generation must be reduced. Also, the difference between actual and apparent infestation rates must be minimized. The following recommendations should aid in keeping the problems encountered with sequential sampling for NPTM to a minimum: (1) To reduce variability between apparent infestation rates, stratification of the sampling by tree size can be implemented. Small and large trees can be sampled and an average of the two used or if the trees are mostly of one class a weighted sample between the two classes can be used; (2) Unless needed for absolute population studies, tips should not be collected for X-ray or dissection. The dissection of the tips in the field or the use of published regression relationships may be used to calculate actual infestation rates; (3) Field workers should be trained to distinguish between fresh and old attacks; (4) To obtain a more accurate representation of the NPTM population, the number of trees sampled should be increased but the number of tips sampled on each tree decreased. Since NPTM show aggregate tendencies by whorl, sampling one tip per whorl will not significantly reduce accuracy (Hedden 1979). If these recommendations were implemented, then sequential sampling for the NPTM could be a valuable tool. Once the problem of rapidly and accurately estimating NPTM populations is solved, then the construction of economic threshold levels for the NPTM can be initiated.

Acknowledgment

This research was supported by McIntire-Stennis funds and a grant from Sigma Xi.

References Cited

Berisford, C. W. 1974. Comparisons of adult emergence periods and generations of the pine tip moths, *Rhyacionia frustrana* and *R. rigidana*. Ann. Entomol. Soc. Am. 67: 666–668.

Berisford, C. W., and H. M. Kulman. 1967. Infestation rates and damage by the Nantucket pine tip moth in six loblolly pine categories. For. Sci. 13: 428–438.

1969. Whole-tree and top-level populations of the Nantucket pine tip moth not correlated. J. Ga. Entomol. Soc. 4: 142–144.

Eikenbary, R. D., and R. C. Fox. 1968. Arthropod predators of the Nantucket pine tip moth, *Rhyacionia frustrana*. Ann. Entomol. Soc. Am. 61: 1218–1221.

Fox, R. C., and E. W. King. 1963. A sampling technique for the Nantucket pine tip moth, *Rhyacionia frustrana* (Comst.). S.C. Agric. Exp. Stn., Entomol. Zool. Res. Ser. 60.

Garguillo, P. M., and C. W. Berisford. 1981. Sampling for pine tip moths a procedural guide. Univ. Ga. Agric. Exp. Stn. Bull. No. 272.

Garguillo, P. M., C. W. Berisford, and L. V. Pienaar. 1983. Two-stage cluster sampling for pine tip moths. Environ. Entomol. 12: 81–90.

Hedden, R. L. 1979. Sampling methods for tip moth in loblolly pine plantations. Weyerhauser Corp. So. For. Res. Cent., For. Res. Tech. Rep.

Johnson, P. C. 1977. Sex-ratio estimation, sequential sampling, and the programmable calculator. Bull. Entomol. Soc. Am. 23: 251–254.

Kuno, E. 1969. A new method of sequential sampling to obtain the population estimates with a fixed level of precision. Res. Popul. Ecol. 11: 127–136.

Lashomb, J. H., and A. L. Steinhauer. 1975. Nantucket pine tip moth damage in Maryland as influenced by moth density, host preference, generation differences and life history. Md. Agric. Exp. Stn. MP 857.

Lashomb, J., A. L. Steinhauer, and G. Dively. 1980. Comparison of parasitism and infestation of Nantucket pine tip moth in different aged stands of loblolly pine. Environ. Entomol. 9: 397–402.

Stephen, F. M., and G. W. Wallis. 1978. Tip damage as an index of Nantucket pine tip moth population density (Lepidoptera: Tortricidae). Can. Entomol. 119: 917–920.

Wakely, P. C. 1935. Notes on the life cycle of the Nantucket tip moth *Rhyacionia frustrana* Comst. in southeastern Louisiana. U.S. Dep. Agric. For. Ser., So. For. Exp. Stn. Occ. Pap. No. 45.

Yates, H. O. 1960. The Nantucket pine moth *Rhyacionia frustrana* Comst.: a literature review. U.S. Dep. Agric. For. Serv., S.E. For. Exp. Stn. Pap. No. 115.

Received for publication 8 March 1984; accepted 25 July 1984.

Table 3. Number of tips and number of infested tips by generation for all trees on Site I

Tree section	No. tips infested/total tips					
	Overwinter	Generation				
		I	II	III	IV	V
1	15/ 92	5/ 50	8/ 40	20/ 46	18/ 26	7/ 21
2	17/ 191	6/ 70	18/ 109	26/ 73	30/ 62	11/ 40
3	48/ 946	12/201	36/ 563	311/1,087	130/ 862	99/ 707
4	12/1,038	11/319	19/ 343	178/ 716	70/ 614	28/ 442
5	1/ 134	1/ 34	1/ 29	5/ 64	1/ 39	1/ 14
Totals	93/2,041	35/674	82/1,086	540/1,986	249/1,603	146/1,224

resulted in the trees not producing very many new tips during the growing season. This led, in turn, to a decrease in available tips for the NPTM, thus reducing the infestation rate. Lashomb et al. (1980) suggested that increased branching (i.e., more tips) on the lower whorls enables pines to escape NPTM infestation by diluting the effects of the infestation. The increased tip production may explain the continued growth and good form of the loblolly pines as opposed to the stunted appearance of the shortleaf pines. The loblolly pines were producing more new tips, especially in the lower whorls, while the shortleaf pines were not (Tables 3 and 4).

The variation in the apparent infestation rates within the generations was due primarily to varied size and height distribution of trees sampled. No sampling stratification by tree size was incorporated in the sampling scheme. On both sites trees sampled ranged from less than 30 cm up to 300 cm. The variation in tree heights gave a wide range in the number of tips sampled on each tree and contributed to the variation of $\hat{P}_a$ within the same generation (Tables 1 and 2).

The differences between the apparent infestation rate and the actual infestation rate (Fig. 1 and 2) were probably caused by two factors: difficulty in distinguishing between fresh attacks and attacks by earlier generations, which became increasingly difficult as the season progressed; and attack of the same tips by the NPTM, causing the larvae to feed and pupate further down in the tip, thus causing them to be missed because not enough of the tip was collected and examined for pupae.

Many researchers (Fox and King 1963, Eikenbary and Fox 1968, Berisford and Kulman 1969, Stephen and Wallis 1978, Hedden 1979) noted that the upper whorls have a higher proportion of infested tips. Results from this study support this conclusion. On Site I, section 1 had $\hat{P}_a = 0.24$ and $\hat{P}_A = 0.19$, while section 2 had $\hat{P}_a = 0.23$ and $\hat{P}_A = 0.20$. Section 3 had $\hat{P}_a = 0.22$ and $\hat{P}_A = 0.10$. Section 4 had $\hat{P}_A = 0.03$ and $\hat{P}_a = 0.04$, while section 5 had $\hat{P}_a = 0.03$ and $\hat{P}_A = 0.04$. Site II had $\hat{P}_a = 0.44$ and $\hat{P}_A = 0.40$ for section 1. Section 2 had $\hat{P}_a = 0.26$ and $\hat{P}_A = 0.24$. Section 3 had $\hat{P}_a = 0.21$ and $\hat{P}_A = 0.20$. Section 4 had $\hat{P}_a = 0.14$ and $\hat{P}_A = 0.13$. Section 5 had $\hat{P}_a = 0.05$ and $\hat{P}_A = 0.04$.

Although sections 1 and 2 had higher proportions of infested tips, they did not contain as many total tips (Tables 3 and 4). Berisford and Kulman (1967) noted the economic importance of the terminal leader and first laterals in terms of economic growth. If the terminal leader and top whorl are continually attacked, then growth can be seriously retarded.

We found highly significant correlations ($P \leq 0.001$) between $\hat{P}_a$ and $\hat{P}_A$ for shortleaf pine (Site I) and loblolly pine (Site II), and for both species combined (Table 5) for all generations analyzed separately and combined. Coefficients of determination (r^2) were 0.85 for Site I, 0.87 for Site II, and 0.82 for both sites combined. Stephen and Wallis (1978) found a highly significant correlation between apparently infested tips and actually infested tips on shortleaf pine in Arkansas ($r^2 = 0.74$).

The linear relationship between apparently and actually infested tips can be used for sampling NPTM. If regressions are formulated for pine species with varying site and stand conditions, then apparently infested tips can serve as an index for actually infested tips with reasonable accuracy. Se-

Table 4. Number of tips and number of infested tips by generation for all trees on Site II

Tree section	No. tips infested/total tips				
	Overwinter	Generation			
		I	II	III	IV
1	22/ 17	9/ 24	17/ 36	8/ 21	20/ 35
2	51/ 330	17/ 79	21/ 67	28/ 93	46/ 95
3	75/1,072	67/287	145/ 585	143/ 605	177/ 541
4	69/1,690	41/520	160/ 846	158/ 935	223/1,063
5	4/ 179	2/ 47	5/ 61	5/ 103	1/ 66
Totals	221/3,288	136/957	348/1,595	342/1,757	467/1,800

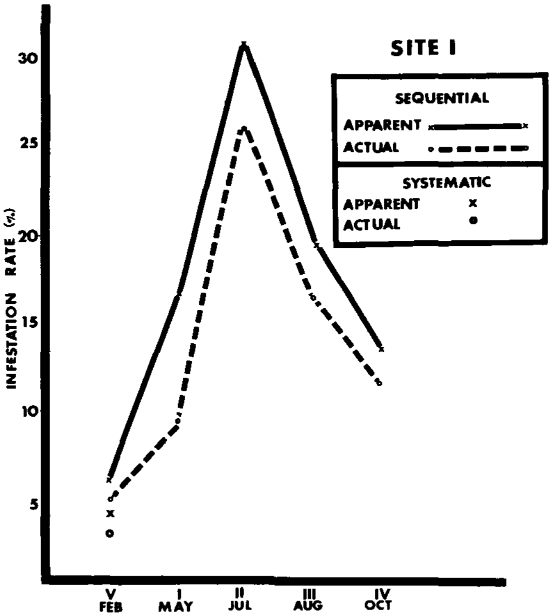

Fig. 1. Apparent and actual combined infestation rates for the Nantucket pine tip moth for Site I.

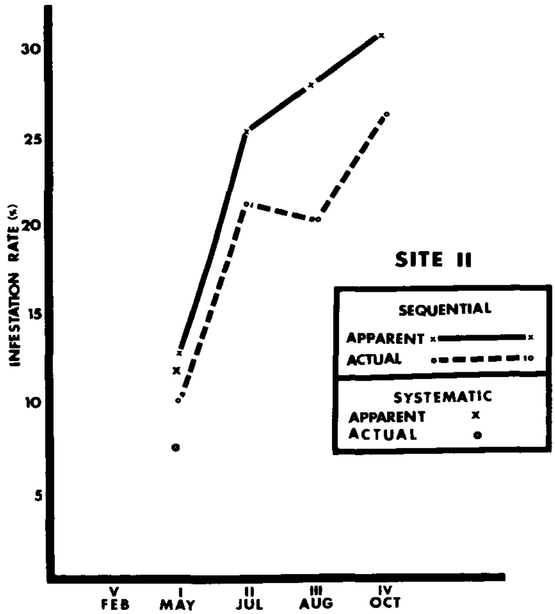

Fig. 2. Apparent and actual combined infestation rates for the Nantucket pine tip moth for Site II.

for generation V before overwintering as pupae. Emergence from the overwintering generation occurred the following March. Since the life stages were synchronous, only one species of *Rhyacionia* was assumed to be present (as previously noted by Berisford [1974]).

Baseline data for Site I (Table 1) was collected during the 1978–1979 overwintering generation and during the first generation of 1979 for Site II (Table 2). Apparent and actual infestation rates were estimated by sequential sampling for the overwintering generation of 1978–1979 on Site I (Table 1) and the first four generations of 1979 for Site I (Table 1) and Site II (Table 2). Tables 1 and 2 also provide the $\hat{P}_a$ and $\hat{P}_A$ for all the sequential sampling episodes combined.

Site I had $\hat{P}_a$ ranging from 0.03 to 0.41 (Table 1), with the highest combined $\hat{P}_a$ occurring during generation II. Site II had $\hat{P}_a$ ranging from 0.02 to 0.44 (Table 2), with the highest combined infestation rate occurring during generation IV. The combined $\hat{P}_a$ for the overwintering generation on Site I was 0.06 and 0.04 for the sequential samples and the systematic sample, respectively (Table 1). A high rate of overwintering mortality occurred during the winter of 1978–1979.

The combined $\hat{P}_a$ for generation I on Site I was 0.16 (Table 1, Fig. 1), approximately 10% higher than the overwintering generation. One decision ($\hat{P}_a = 0.33$) was considerably higher than the other four decisions, due to a large tree sampled near the study site perimeter, with a high NPTM infestation rate. In general, pines on the study site perimeter had higher infestation rates. This non-random distribution of infested trees has not been incorporated in the sampling scheme.

The combined $\hat{P}_a$ for generation II on Site I was 0.30, or approximately 14% higher than the first generation (Table 1). One decision ($\hat{P}_a = 0.41$) was considerably higher than the others, due to perimeter effect. The infestation rate peaked in generation II (Fig. 1) and declined in subsequent generations on Site I.

The combined $\hat{P}_a$ values on Site II for generation I were 0.13 and 0.11 for the sequential sampling and the systematic sampling, respectively. The combined $\hat{P}_a$ on Site II for generation II was 0.25, or almost twice the rate for generation I and it continued to increase through generation IV (Fig. 2). Lashomb and Steinhauer (1975) noted a similar increase in infestation during the period when loblolly pine was putting out new growth, providing an increase in available tips for the NPTM. The period between generation I and generation II had the greatest increase in infestation rate, for both the loblolly and shortleaf pine.

The reasons for the peak infestation in generation II for Site I and the continued increase in infestation for Site II were apparently due to different site factors. Pines on Site I had poor form and appearance compared to Site II trees. Site I had many deeply eroded gulleys running through it, with large areas washed out. Yates (1960) noted that erosion increased the severity of NPTM attack and sometimes led to NPTM-caused mortality. The pines on Site I had increasing numbers of dead tips as the season progressed. The effects of the NPTM attack, combined with poor site quality,

Table 1. Actual (±SE) and apparent (±SE) estimated infestation rates for Nantucket pine tip moths by generation on Site I

Sample type	Generation	Actual rate $\hat{P}_A \pm$ SE	Apparent rate $\hat{P}_a \pm$ SE
Systematic	Overwintering	0.02 ± 0.01	0.04 ± 0.02
Sequential	Overwintering	0.05 ± 0.02	0.06 ± 0.02
		0.07 ± 0.02	0.08 ± 0.02
		0.03 ± 0.03	0.04 ± 0.03
		0.07 ± 0.02	0.08 ± 0.02
		0.03 ± 0.03	0.03 ± 0.03
		0.05 ± 0.02	0.08 ± 0.02
	Combined	0.05 ± 0.01	0.06 ± 0.01
Sequential	Generation I	0.06 ± 0.02	0.08 ± 0.02
		0.05 ± 0.02	0.33 ± 0.02
		0.05 ± 0.02	0.07 ± 0.02
		0.13 ± 0.02	0.15 ± 0.02
		0.13 ± 0.03	0.18 ± 0.03
	Combined	0.08 ± 0.02	0.16 ± 0.05
Sequential	Generation II	0.20 ± 0.03	0.24 ± 0.03
		0.22 ± 0.03	0.27 ± 0.03
		0.35 ± 0.03	0.41 ± 0.03
		0.20 ± 0.02	0.24 ± 0.02
		0.29 ± 0.03	0.32 ± 0.03
	Combined	0.25 ± 0.03	0.30 ± 0.04
Sequential	Generation III	0.17 ± 0.02	0.20 ± 0.02
		0.15 ± 0.03	0.18 ± 0.03
		0.13 ± 0.02	0.16 ± 0.02
		0.17 ± 0.02	0.22 ± 0.02
		0.18 ± 0.03	0.21 ± 0.03
	Combined	0.16 ± 0.04	0.19 ± 0.01
Sequential	Generation IV	0.14 ± 0.02	0.15 ± 0.02
		0.08 ± 0.02	0.09 ± 0.02
		0.13 ± 0.03	0.14 ± 0.03
		0.14 ± 0.02	0.17 ± 0.02
		0.09 ± 0.03	0.11 ± 0.03
	Combined	0.12 ± 0.03	0.13 ± 0.01

Table 2. Apparent (±SE) and actual (±SE) infestation rates of the NPTM by generation on Site II

Sample type	Generation	Actual rate $\hat{P}_A \pm$ SE	Apparent rate $\hat{P}_a \pm$ SE
Systematic	Generation I	0.13 ± 0.04	0.11 ± 0.05
Sequential	Generation I	0.00 ± 0.03	0.02 ± 0.03
		0.13 ± 0.02	0.16 ± 0.02
		0.14 ± 0.02	0.19 ± 0.02
		0.03 ± 0.03	0.04 ± 0.03
		0.20 ± 0.03	0.23 ± 0.03
	Combined	0.10 ± 0.04	0.13 ± 0.04
Sequential	Generation II	0.30 ± 0.03	0.34 ± 0.03
		0.23 ± 0.03	0.23 ± 0.03
		0.20 ± 0.02	0.27 ± 0.02
		0.18 ± 0.03	0.23 ± 0.03
		0.16 ± 0.03	0.20 ± 0.03
	Combined	0.21 ± 0.02	0.25 ± 0.02
Sequential	Generation III	0.24 ± 0.02	0.40 ± 0.02
		0.16 ± 0.02	0.21 ± 0.02
		0.17 ± 0.03	0.27 ± 0.03
		0.19 ± 0.03	0.29 ± 0.03
		0.20 ± 0.03	0.24 ± 0.03
	Combined	0.19 ± 0.01	0.28 ± 0.02
Sequential	Generation IV	0.25 ± 0.02	0.30 ± 0.02
		0.17 ± 0.02	0.29 ± 0.02
		0.38 ± 0.03	0.44 ± 0.03
		0.29 ± 0.03	0.30 ± 0.03
		0.19 ± 0.02	0.20 ± 0.03
	Combined	0.26 ± 0.04	0.31 ± 0.04

a sequential sampling routine developed by Johnson (1977) for an HP-65 programmable calculator. A "random walk" was taken from the study site center and at each change of direction the tree closest to that point was sampled for NPTM. The total number of tips and the number of apparently infested tips on the sampled tree were entered into the sampling routine. If the precision criteria were not met, a new random distance and direction were taken from the current sampling point and a new tree selected. If the specified point fell outside the study area, a new random distance and direction were applied from the previous sampling point.

When a sufficiently precise estimate was reached sampling ceased. The sampling criterion was $\hat{P}_a \pm 0.05$ with a 95% confidence limit. Sequential sampling estimates of $\hat{P}_a$ were made five times at each site (six for the overwintering generation on Site I) for each NPTM generation and compared to baseline systematic sampling for accuracy.

All apparently infested tips and a random sample of apparently uninfested tips, as described for the baseline data, were taken to the laboratory for analysis.

Collected tips were stored at approximately 5°C to halt NPTM development. Radiographs were obtained in a Faxitron Model 43805 dual cabinet X-ray machine, using Kodak M-K Industrial film. Tips were exposed for 20 s at 50 kV potential at a distance of 115 cm, and radiographs were examined for life stages of NPTM present. When terminal bud clusters were too dense for adequate interpretation using radiographs, the tips were dissected to determine accurately if they were infested by NPTM.

Actual infested tips were summarized by section, tree, and sampling episode. A simple linear regression was used to examine the relationship between apparent and actual infestation ratio, and between whole-tree populations and populations in sections 1 and 2.

Results and Discussion

In 1979, the Nantucket pine tip moth completed five successive nonoverlapping generations, excluding the 1978–1979 overwintering generation. Although four generations per year often occur in Texas (Wakely 1935), a fifth generation may occur with favorable weather conditions (Yates 1960). The length of each generation varied from 2 months for generation I to 1.5 months for generations II, III, and IV, and approximately 1 month

Estimating Infestation Rates of the Nantucket Pine Tip Moth (Lepidoptera: Tortricidae) Through Sequential Sampling

BERNARD C. ANDERSEN,[1] DAVID L. KULHAVY, AND PAUL C. JOHNSON[2]

School of Forestry, Stephen F. Austin State University, Nacogdoches, Texas 75962

Environ. Entomol. 13: 1593–1597 (1984)

ABSTRACT Sequential sampling to estimate proportion from a binomially distributed population was used to estimate apparent and actual infestation rates of the Nantucket pine tip moth, *Rhyacionia frustrana* (Comstock), on loblolly, *Pinus taeda*, and shortleaf pine, *P. echinata*. Populations of *R. frustrana*, as measured by infested tips, increased from the overwintering generation through the second generation (July), then decreased rapidly for *P. echinata*. Populations of *R. frustrana* continued to increase through the season in *P. taeda*. Tip moth infestations were concentrated in the top one-half of the tree.

MOST SURVEYS to characterize Nantucket pine tip moth (NPTM), *Rhyacionia frustrana* (Comstock), populations are performed by counting the number of damaged (i.e., apparently infested) tips in the terminal and top whorl on a predetermined number of trees (Fox and King 1963, Hedden 1979, Gargiullo and Berisford 1981, Gargiullo et al. 1983). The correlation between infested tips in the top whorl and infested tips in the whole tree, however, is unreliable (Berisford and Kulman 1967) and depends on tree age and infestation rate (Stephen and Wallis 1978).

This study applies a sequential sampling approach developed to estimate proportions from binomially distributed populations with a predetermined precision as modified by Johnson (1977) for use with a programmable calculator. Johnson's (1977) routine also used precision levels based on confidence limits rather than the standard error/mean ratios used by Kuno (1969). The relationship between apparently infested tips and the actual infestation rates was also examined. The two assumptions associated with this type of sequential sampling are: samples are drawn at random without replacement, and the population size is known or may be estimated (Johnson 1977). Since an unrestricted random sample of tips was not practical, trees were selected at random and all tips on selected trees sampled for tip moth infestation. Error in estimating population size is critical only when the total sample size approaches the population size and was not a problem in this study.

Materials and Methods

Tip moths were sampled in naturally seeded stands of shortleaf, *Pinus echinata* Mill., (Site I) and loblolly pine, *Pinus taeda* L., (Site II) in Nacogdoches, Nacogdoches Co., Texas. Site I, a 1.24-ha tract, is located 0.01 km south of the intersection of Loop 224 and Highway 21 in Nacogdoches. Regeneration of the shortleaf pine is of mixed age (1–15 years) and is patchy because some areas are unable to sustain seedling growth due to erosion and soil washout. The terrain is level to gently sloping (0–4% slope). The soil is an eroded deep fine loamy sand, mainly of the Darco series.

Site II, a 1.05-ha tract, is located 0.01 km east of the intersection of University Drive and F.M. 1878 in Nacogdoches. Regeneration of the loblolly pine is patchy; tree ages vary from 1 to approximately 10 years. The terrain is level to gently sloping (0–4% slope). The soil is a fine loamy sand with the Trawick, Attoyac, and Nacogdoches series present. To determine infestation levels that would accurately characterize NPTM population levels at the beginning of the sampling season, baseline data were collected for each site on 0.02-ha quadrats systematically located to sample approximately 5% of the stand. Random numbers were selected to generate distances and directions from each quadrat center to locate 2-m-square (2 m^2) subplots. All trees within the 2 m^2 subplots were sampled for NPTM. Each tree was divided into five sections: (1) the terminal leader, (2) the remainder of the top whorl, (3) the top one-half of the crown minus the top whorl, (4) the lower one-half of the crown, and (5) interior tips originating from the main stem. Collected tips were stripped of needles and taped to sheets of paper and X-rayed or dissected to check for NPTM infestation. Apparent infestation rates ($\hat{P}_a$) obtained from field observations were compared to actual infestation rates ($\hat{P}_A$) obtained from X-ray and dissection. All tips from the first two sections were collected for X-ray. All apparently infested and a random sample of apparently uninfested tips were collected from sections 3 through 5.

Infestation rates of NPTM were estimated using

[1] Current address: Oklahoma Forestry Div., Box 368, Talihina, OK 74571.

[2] Current address: Entomology Dept., Univ. of New Hampshire, Durham, NH 03824.