AN EXAMINATION OF SIZE AND OVERWINTERING BEHAVIOR CRITERIA USED FOR SEPARATING *RHYACIONIA FRUSTRANA* (COMSTOCK) FROM *RHYACIONIA BUSHNELLI* (BUSCK) (LEPIDOPTERA: TORTRICIDAE)

by

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INTRODUCTION

Importance

The genus Rhyacionia (Lepidoptera: Tortricidae, Olethreutinae) contains approximately 35 species worldwide (Powell and Miller 1978). Larvae feed in the new growth of pine. Some species are of particular importance in nurseries, ornamental plantings, shelterbelts and reforestation projects where severe infestations may result in deformed and slow-growing trees (Powell and Miller 1978, Graham and Baumhofer 1927). Young pines are most susceptible to damage since moths rarely infest the terminal shoots of trees over 12 feet tall.

Powell and Miller (1978) have summarized some of the biological information concerning taxa of Rhyacionia. In general, adults emerge in the spring and deposit eggs either on the needles, needle sheaths, bud or shoot tip. First and second instar larvae usually mine in the needle prior to entering the bud or shoot. Later instar larvae feed within the shoot and pupate in the larval tunnel, a hollow bud or on the ground under the tree. The number of generations per year varies with the species and geographical location (Powell and Miller 1978).

Three species of Rhyacionia have been reported in Kansas: the European pine shoot moth, R. buoliana (Heinrich), the Nantucket pine tip moth, R. frustrana (Comstock) (Cooperative

Economic Insect Report 1960, 1961), and the western pine tip moth, R. bushnelli (McKnight 1973). There is some doubt whether both R. frustrana and R. bushnelli are present because of difficulties in separating the species using Miller's criteria (Miller 1967).

Also, there is a good possibility two other Rhyacionia species may occur in Kansas although they have not been officially reported. Since R. rigidana (Fernald) has been detected in counties scattered throughout the southern two-thirds of Missouri (Dickerson and Kearby 1972), its occurence in southeastern Kansas appears likely. The southwestern pine tip moth, R. neomexicana (Dyar) is found throughout the southwestern United States and has been collected in Colorado and Nebraska (Jennings 1975). This species may occur in western Kansas.

Review of Literature

Species determination of pine tip moths in Kansas has become confused because of problems in separating Nantucket pine tip moth, Rhyacionia frustrana (Comstock), from western pine tip moth, Rhyacionia bushnelli (Busck). They have been alternately described as separate species and races within one species (Miller 1967, Heinrich 1923, and Busck 1914). At present they are considered valid species based on differences in size, overwintering behavior and geographical distribution (Miller 1967). No morphological differences between the two species have been found (Heinrich 1923, Miller 1967).

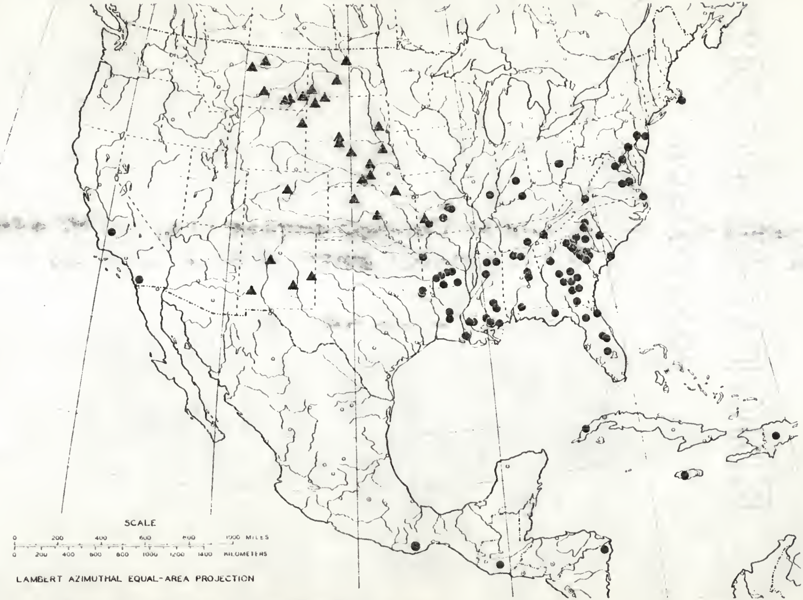
Busck (1914) based his separation of R. bushnelli from R. frustrana on differences in size, details of ornamentation and damage. His distinction based on the latter two criteria is unclear because he did not describe color patterns or the damage. Since no mention is made concerning overwintering behavior, his differentiation must have been made on size alone. Even though Heinrich (1923) considered R. bushnelli to be a "food plant race" of R. frustrana, he retained the name R. frustrana bushnelli as a "racial designation" primarily because of its economic importance. Miller (1967) believes R. bushnelli is more likely a separate species native to its range.

Rhyacionia frustrana was first observed on Nantucket Island in 1876 (Packard 1890). Rhyacionia bushnelli was discovered in 1909 in Nebraska and was reported in New Mexico almost simultaneously (Swenk 1927, Busck 1914). The distribution of the two species in the United States is shown in Plate I. There are several theories as to how R. bushnelli was introduced into Nebraska. Some believe the infestation was a result of bringing either jack pine seedlings from Minnesota or western yellow pine seedlings from the Black Hills (Graham and Baumhofer 1927, Swenk 1927). The source of the Minnesota and Black Hills infestations is not known. Rhyacionia frustrana is not believed to occur in the Lake States or Black Hills

EXPLANATION OF PLATE I

Distribution records for *Rhyacionia frustrana* and *R. bushnelli* in the United States (From Powell and Miller 1978) (Used by permission from Dr. W. E. Miller).

PLATE I



R. frustrana- ● R. bushnelli- ▲

region (Miller 1967).

In addition to geographical ranges, the overwintering habit of the two species differ. Although both overwinter as pupae, R. bushnelli larvae drop to the ground (Graham and Baumhofer 1927) while R. frustrana larvae remain in the shoots to pupate. Both species spend the non-overwintering pupal stages in the shoots (Miller 1967).

According to Miller (1967), the remaining criterion for separation is forewing length. Forewing length of R. bushnelli ranges from 5.0 to 7.5 mm (118n) for males and 5.0 to 8.0 mm (102n) for females. Forewing length of R. frustrana ranges from 4.0 to 7.0 mm (103n) for males and 4.0 to 7.5 mm (108n) for females (Powell and Miller 1978).

No one has investigated mechanisms other than morphological differences which may reproductively isolate sympatric populations, if such exist, of R. frustrana and R. bushnelli. Such mechanisms may include hybrid inviability, specific female sex pheromones or mating behavior. Berisford (1974b) investigated possible mechanisms for sexual isolation between sympatric populations of R. frustrana and R. rigidana. He found morphological differences in genitalia, different female sex pheromones and reciprocal inhibition of male response may isolate the two species.

Based on size, tip moths in Kansas have been identified as R. bushnelli (McKnight 1973, W. E. Miller, personal communication with H. E. Thompson 1975). However, they would be identified as R. frustrana using the overwintering criterion.

In this thesis, Rhyacionia species designations will follow previously reported identifications.

It was the objective of this research to:

1. Determine size variability of field-collected R. frustrana and R. bushnelli from populations in Georgia, Oklahoma, Kansas and Nebraska.
2. Rear R. frustrana and R. bushnelli on Pinus ponderosa and P. sylvestris seedlings under controlled conditions for examining the validity of using size as a criterion for species separation.
3. Investigate the overwintering potential of Kansas Rhyacionia at Halsey, Nebraska.
4. Obtain an estimate of temperature differences, if such exist which determine whether the pupae of these species overwinter in the shoots or on the ground.
5. Determine if male Rhyacionia in Kansas are attracted to the female sex pheromone of R. frustrana.

MATERIALS AND METHODS

Study Areas

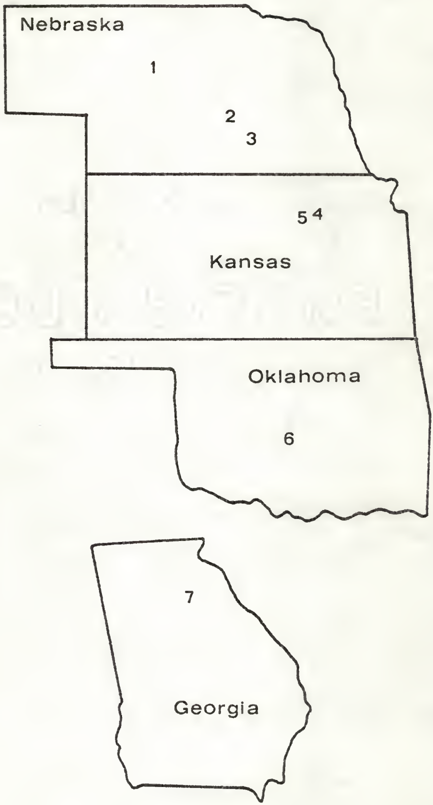
Observations and collections of the two species were made at locations in Nebraska, Kansas and Oklahoma (PlateII). Collections were made from ponderosa pine, Pinus ponderosa var.

EXPLANATION OF PLATE II

The numbers 1-7 denote locations where collections or observations of *Rhyacionia* were made.

1. *Pinus ponderosa* plantings in the Nebraska National Forest, 2 mi. W of Halsey, Nebraska on State Highway 2.
2. *Pinus ponderosa* shelterbelt about 4 mi. NW of Cairo, Nebraska on State Highway 2.
3. *Pinus ponderosa* provenance planting about 4 mi. E of Hastings, Nebraska on U.S. Highway 6.
4. *Pinus sylvestris* Christmas tree plantings in Riley County, Kansas.
5. *Pinus ponderosa* provenance planting about 3 mi. N of Junction City, Kansas on State Highway 57.
6. *Pinus ponderosa*, *P. sylvestris* and *P. taeda* plantings at the Oklahoma State Nursery at Goldsby, Oklahoma.
7. *Pinus taeda* plantations in Clarke County, Georgia.

PLATE II



scopulorum Englem.; Scotch, P. sylvestris L.; and loblolly, P. taeda. Rhyacionia frustrana pupae from P. taeda in Georgia were provided by Dr. C. W. Berisford, Associate Professor of Entomology, University of Georgia, Athens, Georgia.

Biological Study

Field Collections. Forewing length and pupal weight determinations were made to obtain size estimates of males and females obtained from field infestations because Miller (1977) showed forewing length accounts for 82-87% of variation in biomass among olethreutid moths.

Tip moth pupae were collected from ten-year-old P. ponderosa at Halsey, NB., Hastings, NB., and Junction City, KS.; four to six-year-old P. sylvestris at Manhattan, KS.; two-year-old P. ponderosa and P. sylvestris and four to six-year-old P. taeda at Goldsby, OK.; and two to five-year-old P. taeda at Athens, GA.

In the laboratory, pupae were removed from infested shoots, sexed (Yates 1969) and held individually in 5 cm petri dishes for daily weight determinations. Pupae were kept in a growth chamber at a temperature of approximately 26° C. and a relative humidity of 50-75%.

Measurements. Weight determinations were made using either a Type H20 Mettler analytical balance (.01 mg sensitivity) or Cahn Electrobalance (.001 mg sensitivity). Pupal weight recorded on the day prior to moth emergence was selected to establish a standard time of measurement for basing size comparisons between Rhyacionia populations sampled.

Forewing length (including fringe but excluding tegula) was measured to the nearest .1 mm with the aid of an ocular micrometer in a Bausch and Lomb binocular microscope.

Rearing. Rhyacionia frustrana and R. bushnelli larvae were reared on two-year-old seedlings of P. sylvestris and P. ponderosa grown in either one-half or one gallon containers.

Several methods were employed in this study to obtain R. frustrana for rearing purposes. In the laboratory, adults or pupae were placed in screen-glass cages (measuring 51 x 43 x 48 cm) containing clipped P. ponderosa and P. sylvestris shoots. The cut ends of these shoots were kept in water. A flourescent light source was placed above each cage providing eight hours light and fresh shoots were placed in the cages as needed. Needles with eggs were removed, placed in plastic boxes (measuring 13 x 3.5 x 13.5 cm)[1] containing moistened filter paper, and kept in a growth chamber.

Also in the laboratory, screen cages (measuring 7.5 x 2.8 x 3.2 cm) were placed around the shoot tips of P. sylvestris and P. ponderosa seedlings. Adults were then placed inside and the seedlings were placed in a growth chamber having a temperature of approximately 28° C. with a 15:9 light-dark cycle. Following a 5-6 day period for mating and oviposition, seedlings were

[1]Althor Products, 202 Bay 46th Street, Brooklyn, N.Y.

placed in a growth chamber providing a temperature of approximately 26° C. and 50-75% relative humidity. Shoots were inspected frequently for eggs and larvae.

Other methods used to obtain larvae involved placing pupae or adults from Athens, GA. in screen cages located either outside or in a greenhouse. In the greenhouse, approximately 150 clipped P. ponderosa shoots (15-20 cm) were placed in a cage (measuring 2.2 x .9 x 1.2 m) prior to adding pupae and adults. These shoots were inserted through holes in 1.3 cm styrofoam into water contained in 5.0-7.5 cm deep aluminum pans. Shoots were examined for larvae approximately seven days later. Two cages (measuring 1.5 x .6 x .6 m) were used outside. Approximately 30 P. ponderosa seedlings were placed in each cage. Seedlings were inspected daily for larvae after five days. Rhyacionia frustrana larvae were also obtained by collecting infested P. taeda shoots at Goldsby, OK. Rhyacionia bushnelli were obtained as eggs or first instar larvae from P. ponderosa at Junction City, KS.

Larvae obtained for rearing were detected by the appearance of a short yellow streak on infested needles or by the presence of frass or resin in the area of the bud or needle base. In the laboratory, first instar larvae were removed from the needle or shoot and placed on the needle or bud of a P. ponderosa or P. sylvestris seedling. Each seedling was infested with one larva and inspected daily for larval establishment. Seedlings not initially infested were reinfested. Seedling height, shoot length and shoot diameter were determined prior to being infested.

Rhyacionia frustrana larvae obtained using adults from Georgia as parent stock were reared on P. ponderosa seedlings. Larvae collected in Oklahoma were used to infest P. sylvestris seedlings.

Infested seedlings were held in growth chambers having a temperature of 26° C., a relative humidity of 50-75% and a 15:9 light-dark photoperiod. Approximately two weeks after infestation, a plastic box (measuring 3.8 x 3.2 x 5 cm) was placed around the infested tip of each seedling to prevent emerging moths from escaping.

The date of adult emergence and the amount of shoot dieback caused by larval feeding was recorded. Forewing length measurements were made using methods previously described.

Three trials were conducted with 50 individuals of each tip moth species per one Pinus species making one trial. Two trials were conducted with each Rhyacionia species on P. ponderosa. Due to the unavailability of growth chamber area and R. frustrana larvae, only one trial using P. sylvestris was completed.

Overwintering Study. To investigate the overwintering potential of Kansas tip moths in Nebraska, approximately 125 shoots from the Junction City, KS. planting containing overwintering pupae were attached to the shoots of pine at Halsey, NB.

on November 14, 1977. On April 6, 1978, the shoots were collected and returned to Manhattan, KS. The shoots were placed in a screen cage (measuring 1.5 x .6 x .6 m) located outside. A few days prior to the retrievel of shoots from Halsey, about 100 infested shoots were collected from Junction City and placed in a separate screen cage. On June 1, 1978, the contents of each cage was examined for moths. Also in March, additional infested shoots were collected from Junction City in an attempt to estimate pupal mortality prior to moth emergence. Pupae were removed from these shoots and examined. Approximately 130 of these pupae were placed in a refrigerator and held until June 1, 1978.

Overwintering behavior of tip moths at Cairo, NB. and at Halsey, NB. was studied by collecting soil from under five infested trees and by examining approximately 100 infested shoots at each location. Shoots were examined for pupae on April 6, 1978 and September 25 and 26, 1978. In November 1977, soil was collected under five infested trees at Junction City. Soil samples were brought to the laboratory and sifted for pupae.

On December 15, 1977, a CR5 Digital Thermograph was taken to the Junction City plantation to monitor ambient and soil temperatures. Maximum and minimum temperatures were recorded each day until March 1, 1978. Unfortunately, similar temperature information could not be obtained at Halsey due to the unavailability of equipment.

Temperature differences between Junction City, KS., Hastings, NB. and Halsey, NB. were examined using United States Weather Service Climatological data from each location and temperatures recorded by the thermograph at Junction City.

Pheromone Trapping. Female sex pheromone of *R. frustrana* and Zoecon traps were provided by Dr. C. W. Berisford. The traps were baited with five female equivalents of extract by pipetting the solution onto a cone-shaped filter paper placed in the bottom of the trap. Traps were hung in trees at the *P. ponderosa* plantation near Junction City, KS. Seven traps were baited just before dark on April 19, April 24, and May 2 in 1977. A control trap was used during each trapping period. Traps were collected the following day and moths were removed and counted.

Statistical Analyses. Where possible, data were subjected to analysis of variance or Duncan's multiple range tests. All significance is reported at the 5% level of probability.

RESULTS AND DISCUSSION

Size Determination of Field-Collected *Rhyacionia*

Halsey. *Rhyacionia bushnelli* at Halsey, Nebraska have two generations annually and overwinter as pupae in cocoons in the soil (Swenk 1927). Miller (1967) reported mean forewing lengths of *R. bushnelli* to be approximately 6.9 mm (22n) for females and

6.6 mm (29n) for males. The majority of these moths were from Montana and South Dakota. A few Nebraska specimens might have been included, but probably not from any further south than Halsey (W. E. Miller, personal communication 1977). In a more recent report, mean forewing lengths have been revised to be approximately 6.5 mm for females and 6.2 mm for males (Powell and Miller 1978). These means are based on a much larger sample from a larger area and are smaller than previously reported.

Mean forewing lengths and pupal weights of R. bushnelli collected at Halsey in 1977 are shown in Table 1. To some extent, differences between our determinations and Miller's may be a result of the way forewing length measurements were made. Miller's determinations were made with the unaided eye, counting divisions (to nearest .5 mm usually, rarely to the nearest .2 mm) of wing length on a rule held along the leading edge of the wing in the same plane as the wing. Our measurements were made with the aid of an ocular micrometer in a binocular microscope.

Comparing these measurements to those made by Miller in 1967 and 1978, it appears R. bushnelli size may increase slightly with increasing latitude throughout its distribution. However, without having more specific information concerning hosts and geographical locations from which moths were collected, this is only speculation.

Table 1. Forewing length and pupal weight data of first generation male and female *Rhyacionia bushnelli* collected from *Pinus ponderosa* at Halsey, Nebraska in 1977.

	Forewing Length (mm)			Pupal Weight (mg)		
Sex	N	Mean[a]	Range	N	Mean[a]	Range
Male	21	5.6 a	4.6-6.3	21	8.58 a	6.78-11.30
Female	27	6.2 b	5.3-7.2	26	12.28 b	6.43-17.90

[a]Means in the same column followed by a different letter are significantly different at the 5% level by F test.

Although considerable overlap exists in the size ranges of males and females collected at Halsey, analysis of forewing length and pupal weight determinations revealed that females were significantly larger than males.

Hastings. Tip moths at Hastings, Nebraska have been identified as *R. bushnelli* (McKnight 1973). However, like tip moths in Kansas, pupae at Hastings overwinter on the tree (R. A. Read, personal communication 1977).

In 1977, first and second generation pupae were collected at Hastings on June 13 and July 29, respectively. Although first generation pupae at Junction City, Kansas were not collected in 1977, second generation pupae were found on July 24. Therefore, it appears that the duration and number of generations at Hastings are similar to those at Junction City.

Forewing length and pupal weight data for males and females of each generation sampled at Hastings are presented in Table 2. The only significant size differences detected were between males and females, and between the mean forewing lengths of first and second generation females. Also, there exists a considerable overlap in the size ranges of males and females.

Comparisons between mean forewing lengths and mean pupal weights of first generation *R. bushnelli* from Hastings and Halsey revealed no significant differences between males or females (Table 3). Thus, if tip moths from these two locations are to be regarded as separate species, then this distinction

Table 2. Forewing length and pupal weight data of first and second generation male and female *Rhyacionia bushnelli* collected from *Pinus ponderosa* at Hastings, Nebraska in 1977.

		Forewing Length (mm)			Pupal Weight (mg)		
Generation	Sex	N	Mean[a]	Range	N	Mean[a]	Range
First	Male	28	5.6 a	4.7-6.4	21	9.58 a	7.52-12.19
	Female	27	6.2 c	5.3-6.7	24	12.78 b	8.25-16.26
Second	Male	20	5.4 a	4.7-5.7	26	8.68 a	5.39-11.89
	Female	20	6.0 b	5.3-6.7	24	12.14 b	8.15-17.09

[a]Means in the same column followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.

Table 3. Forewing length and pupal weight data of first generation male and female *Rhyacionia bushnelli* collected from *Pinus ponderosa* at Hastings, Nebraska and Halsey, Nebraska in 1977.

		Forewing Length (mm)			Pupal Weight (mg)		
Location	Sex	N	Mean[a]	Range	N	Mean[a]	Range
Halsey	Male	28	5.6 a	4.7-6.4	21	9.58 a	7.52-12.19
	Female	27	6.2 b	5.3-6.7	24	12.78 b	8.25-16.26
Hastings	Male	21	5.6 a	4.6-6.3	21	8.58 a	6.78-11.30
	Female	27	6.2 b	5.3-7.2	26	12.28 b	6.43-17.90

[a]Means in the same column followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.

should be made using overwintering behavior and not size as the criterion.

Junction City. The use of both the size and overwintering behavior criteria has confused the identity of tip moths at Junction City, Kansas. Although pupae at Junction City overwinter on the tree like R. frustrana, the adults have been identified as R. bushnelli because of their larger size. Tip moths have three generations annually at this location.

No significant differences in size were found between first and second generation males or females collected at Junction City in 1978 (Table 4). The results again indicate females were significantly larger than males in both generations.

Size comparisons were also made between second generation R. bushnelli in 1977 and 1978 (Table 5). When size of individuals of the same sex were compared, males and females collected in 1978 were significantly larger than those sampled in 1977. Variations in environmental conditions, host physiology or larval density may, in part, have contributed to the observed size differences.

Mean forewing lengths and mean pupal weights of second generation R. bushnelli collected at Junction City in 1977 were also compared with those of second generation R. bushnelli collected at Hastings, NB. in 1977 (Table 6). Rhyacionia bushnelli from Junction City were significantly smaller than those at Hastings. These differences also are not easily

Table 4. Forewing length and pupal weight data of first and second generation male and female Rhyacionia bushnelli collected from Pinus ponderosa at Junction City, Kansas in 1978.

		Forewing Length (mm)			Pupal Weight (mg)		
Generation	Sex	N	Mean[a]	Range	N	Mean[a]	Range
First	Male	21	5.6 a	5.2-6.2	21	8.64 a	6.28-12.82
	Female	21	6.1 b	5.5-7.0	21	11.80 b	7.42-17.90
Second	Male	23	5.6 a	5.2-6.0	22	8.71 a	5.72-11.26
	Female	25	6.3 b	5.7-7.1	24	12.51	8.28-17.72

[a]Means in the same column followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.

Table 5. Forewing length and pupal weight data of second generation male and female *Rhyacionia bushnelli* collected from *Pinus ponderosa* at Junction City, Kansas in 1977 and 1978.

		Forewing Length (mm)			Pupal Weight (mg)		
Year	Sex	N	Mean[a]	Range	N	Mean[a]	Range
1977	Male	31	5.1 a	4.2-5.9	23	7.12 a	4.43-11.35
	Female	20	5.5 b	4.5-6.5	22	8.93 b	4.79-11.98
1978	Male	23	5.6 b	5.2-6.0	22	8.71 b	5.72-11.26
	Female	25	6.3 c	5.7-7.1	24	12.15 c	8.28-17.72

[a]Means in the same column followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.

Table 6. Forewing length and pupal weight data of second generation male and female *Rhyacionia bushnelli* collected from *Pinus ponderosa* at Hastings, Nebraska and Junction City, Kansas in 1977.

		Forewing Length (mm)			Pupal Weight (mg)		
Location	Sex	N	Mean[a]	Range	N	Mean[a]	Range
Hastings	Male	20	5.4 b	4.7-5.7	26	8.68 b	5.39-11.89
	Female	20	6.0 c	5.3-6.7	24	12.14 c	8.25-16.26
Junction City	Male	31	5.1 a	5.2-6.0	23	7.12 a	5.72-11.26
	Female	20	5.5 b	5.7-7.1	22	8.93 b	8.28-17.72

[a]Means in the same column followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.

accounted for. Besides the possible differences in developmental conditions mentioned earlier, P. ponderosa at Hastings, although planted in the same year as P. ponderosa at Junction City, were considerably taller and less heavily infested by pine tip moths. Therefore, although collections were made from the same species of pine, factors such as larval density, host condition and food quality may also influence tip moth development.

Manhattan. Dick (1969) reported much of the biological information concerning tip moths at the Christmas tree plantings located near Manhattan, Kansas. While tip moths from nearby Junction City have been called R.bushnelli, moths collected from P. sylvestris Christmas trees at Manhattan have been identified as R. frustrana (Dick 1969).

Size data of first and second generation R. frustrana collected from P. sylvestris at Manhattan in 1978 is presented in Table 7. No significant differences between individuals of the same sex were found; however, females were significantly larger than males.

Because no significant size differences were found between individuals of the same sex but from different generations in 1978 at Junction City or Manhattan, size data was pooled for each sex by location to make size comparisons between tip moths at these locations. As shown in Table 8, tip moths obtained from P. ponderosa at Junction City were significantly larger than those collected from P. sylvestris at Manhattan.

Table 7. Forewing length and pupal weight data of first and second generation male and female *Rhyacionia frustrana* collected from *Pinus sylvestris* at Manhattan, Kansas in 1978.

		Forewing Length (mm)			Pupal Weight (mg)		
Generation	Sex	N	Mean[a]	Range	N	Mean[a]	Range
First	Male	21	5.1 a	4.6-5.7	20	7.41 a	4.78-9.90
	Female	27	5.7 b	5.2-6.3	25	9.44 b	7.00-12.66
Second	Male	69	5.0 a	4.5-5.8	65	6.59 a	4.08-8.86
	Female	88	5.6 b	4.5-6.5	77	8.97 b	4.70-12.36

[a]Means in the same column followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.

Table 8. Forewing length and pupal weight data of male and female *Rhyacionia* sp. collected from *Pinus ponderosa* and *P. sylvestris* at Junction City, Kansas and Manhattan, Kansas in 1978.

		Forewing Length (mm)			Pupal Weight (mg)		
Host	Sex	N	Mean[a]	Range	N	Mean[a]	Range
P. sylvestris	Male	90	5.1 a	4.5-5.8	85	6.79 a	4.08-8.86
	Female	115	5.6 b	4.5-6.5	102	9.08 b	4.70-12.66
P. ponderosa	Male	44	5.6 b	5.2-6.2	43	8.68 b	5.72-12.82
	Female	46	6.2 c	5.5-7.1	45	11.98 c	7.42-17.90

[a]Means in the same column followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.

Since these two sampling locations are only about 15 miles apart, discrepencies in Rhyacionia size were attributed to different hosts. Thus, the inconsistency in naming tip moths at these and other locations probably is a result of using the size criterion alone.

It must be emphasized that P. sylvestris from which tip moths were collected are under intensive management. These trees are sheared each summer to stimulate the production of lateral shoots and make well-shaped Christmas trees. These shoots are considerably shorter and smaller in diameter in comparison to normal shoot development by unmanaged P. ponderosa or P. sylvestris.

The exact nature by which tip moth size may be influenced by the physiological state of the host is unknown. However, such factors should be considered when using size criterion for identifying species.

Goldsby. At Goldsby, Oklahoma, R. frustrana were collected from P. ponderosa and P. sylvestris seedlings on July 22, 1978 and from P. taeda, which were approximately three to five ft. tall, on August 31, 1978. Moderate infestations were observed on P. ponderosa and P. sylvestris while P. taeda were heavily infested. When collections were made, all stages of R. frustrana could be found making it difficult to determine which generations were sampled.

Table 9 summarizes results of size comparisons between

Table 9. Forewing length and pupal weight data of male and female *Rhyacionia frustrana* collected from *Pinus ponderosa* and *P. sylvestris* seedlings at Goldsby, Oklahoma in 1978.

		Forewing Length (mm)			Pupal Weight (mg)		
Host	Sex	N	Mean[a]	Range	N	Mean[a]	Range
P. ponderosa	Male	21	4.3 a	3.9-4.9	21	5.56 a	3.88-7.45
	Female	22	5.0 b	4.4-5.7	22	8.08 b	4.56-11.27
P. sylvestris	Male	29	4.4 a	3.8-5.1	25	5.51 a	3.34-8.34
	Female	57	5.1 b	4.3-5.7	31	8.25 b	5.45-10.86

[a]Means in the same column followed by a different letter are significantly different at the 5% level by Duncan's multiple range test.

R. *frustrana* collected from *P*. *ponderosa* and *P*. *sylvestris*. No significant differences in size between males or females were detected. However, for each host, females were significantly larger than males.

Similar size data for *R*. *frustrana* collected from *P*. *taeda* is shown in Table 10. Again, females were significantly larger than males.

Athens. *Rhyacionia frustrana* has three generations annually in the Athens, Georgia area (Berisford 1974a). Size determinations were made for pupae and adults collected and sent by Dr. C. W. Berisford in 1977 and 1978. No forewing length determinations were made for second generation (1978) adults. These adults were placed in screen cages outside and were used as parent stock of larvae that were reared on *P*. *ponderosa* and *P*. *sylvestris* seedlings in growth chambers.

Size comparisons were made between first and second generation males and females collected in 1978, and also between second generation males and females collected in 1977 and 1978.

Significant differences in size were detected between first and second generation males and also females collected in 1978 (Table 11). Of the four locations at which two successive generations were sampled, Athens was the only location where both forewing length and pupal weight data indicate significant size differences exist between males and females from different generations.

Table 10. Forewing length and pupal weight data of male and female Rhyacionia frustrana collected from Pinus taeda at Goldsby, Oklahoma.

	Forewing Length (mm)			Pupal Weight (mg)		
Sex	N	Mean[a]	Range	N	Mean[a]	Range
Male	25	4.4 a	4.0-4.7	25	5.15 a	3.76-7.08
Female	34	4.9 b	3.3-5.7	33	7.74 b	2.74-10.92

[a]Means in the same column followed by a different letter are significantly different at the 5% level by F test.

Table 11. Forewing length and pupal weight data of first and second generation male and female *Rhyacionia frustrana* collected from *Pinus taeda* at Athens, Georgia in 1978.

		Forewing Length (mm)			Pupal Weight (mg)		
Generation	Sex	N	Mean[a]	Range	N	Mean[a]	Range
First	Male	31	4.8 a	4.4-5.3	31	6.38 b	4.58-8.08
	Female	28	5.4 b	4.7-6.3	28	9.85 d	6.89-13.48
Second	Male	-	-	-	21	5.49 a	3.02-8.40
	Female	-	-	-	18	7.78 c	5.44-11.44

[a]Means in the same column followed by a different letter are significantly different at the 5% level by Duncan's multiple range test.

As was the case when size comparisons were made between second generation R. bushnelli from different years, second generation R. frustrana collected in 1978 were significantly larger, by sex, than those collected in 1977 (Table 12). Also, in each year, females were significantly larger than males.

Rearing

Rhyacionia frustrana larvae have been reared on artificial diets in the laboratory (Richmond and Thomas 1976, Creswell et al. 1971). However, no attempts have been made to rear larvae on pine seedlings under controlled conditions. Although Heinrich (1923) reported that R. frustrana reared on western pine, Pinus ponderosa, achieved a size similar to R. bushnelli, he made no mention of the number reared or rearing conditions.

Attempts to obtain larvae for rearing by caging moths with clipped shoots in the laboratory or on the shoot tips of seedlings in the growth chamber proved unsuccessful. Although several authors (Richmond and Thomas 1976, Creswell et al. 1971) have reported similar results, Richmond and Thomas (1977) outline methods for obtaining improved mating of R. frustrana in the laboratory.

In the greenhouse, the majority of clipped P. ponderosa shoots dried up after several days possibly because the shoot resin blocked conductive tissues at the cut end and prevented water uptake. Most of the shoots which remained relatively

Table 12. Forewing length and pupal weight data of second generation male and female *Rhyacionia frustrana* collected from *Pinus taeda* at Athens, Georgia in 1977 and 1978.

		Forewing Length (mm)			Pupal Weight (mg)		
Year	Sex	N	Mean[a]	Range	N	Mean[a]	Range
1977	Male	51	4.3 a	3.7-5.0	42	4.78 a	2.62-7.16
	Female	53	4.8 b	3.9-5.5	41	6.16 b	3.21-8.72
1978	Male	-	-	-	21	5.49 b	3.02-8.40
	Female	-	-	-	18	7.78 c	5.44-11.44

[a]Means in the same column followed by a different letter are significantly different at the 5% level by Duncan's multiple range test.

fresh were infested and some larvae were obtained for rearing purposes. Had seedlings been placed within the greenhouse cage, a greater number of larvae might have been obtained.

Most R. frustrana larvae reared on P. ponderosa were obtained from seedlings and adults in the outdoor cages.

In each trial, no significant differences in mean forewing length between R. bushnelli and R. frustrana males or females were observed (Tables 13-15).

Male and female R. frustrana reared on P. ponderosa or P. sylvestris showed a marked increase in size as compared to size estimates obtained for either parents or individuals of the same generation collected from P. taeda at Athens, GA. or Goldsby, OK. First generation female and male R. frustrana at Athens had mean forewing lengths of 5.4 mm (28n) and 4.8 (29n), respectively. Forewing lengths of second generation R. frustrana at Athens were not determined, however, mean pupal weights indicate these individuals were smaller than first generation tip moths. Females and males collected at Goldsby had mean forewing lengths of 4.9 mm (34n) and 4.4 mm (25n), respectively.

Rhyacionia frustrana collected from two-year-old P. ponderosa and P. sylvestris seedlings (Table 9) are considerably smaller than R. frustrana reared in the growth chamber. However, tip moths collected from P. sylvestris seedlings at Manhattan in 1977 had mean forewing lengths of 5.8 mm (28n) for females and 5.0 mm (12n) for males. These size differences probably are

Table 13. Forewing length and development time data of male and female *Rhyacionia frustrana* and *R. bushnelli* reared on *Pinus ponderosa* in Trial I.

			Forewing Length (mm)		Development Time (days) (Larva-Pupae)	
Rhyacionia sp.	Sex	N	Mean[a]	Range	Mean[a]	Range
R. bushnelli	Male	25	5.4 a	4.5-6.1	39 ab	27-50
	Female	15	6.1 b	5.4-6.9	41 b	33-50
R. frustrana	Male	26	5.4 a	4.1-6.1	36 a	27-55
	Female	20	5.8 b	4.9-6.6	39 ab	29-54

[a]Means in the same column followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.

Table 14. Forewing length and development time data of male and female *Rhyacionia frustrana* and *R. bushnelli* reared on *Pinus ponderosa* in Trial II.

Rhyacionia sp.	Sex	N	Forewing Length (mm) Mean[a]	Forewing Length (mm) Range	Development Time (days) (Larva-Pupae) Mean[a]	Development Time (days) (Larva-Pupae) Range
R. bushnelli	Male	17	5.2 a	4.3-6.1	40 a	25-55
	Female	20	5.8 b	5.0-6.7	38 a	29-55
R. frustrana	Male	19	5.3 a	4.6-5.7	37 a	27-48
	Female	20	5.7 b	5.1-6.3	39 a	28-50

[a]Means in the same column followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.

Table 15. Forewing length and development time data of male and female *Rhyacionia frustrana* and *R. bushnelli* reared on *Pinus sylvestris*.

			Forewing Length (mm)		Development Time (days) (Larva-Pupae)	
Rhyacionia sp.	Sex	N	Mean[a]	Range	Mean[a]	Range
R. bushnelli	Male	19	5.3 a	4.3-6.0	34 a	25-46
	Female	21	6.0 b	5.5-6.8	38 b	27-47
R. frustrana	Male	20	5.6 a	4.9-6.2	34 ab	26-43
	Female	22	5.8 b	4.9-6.8	36 ab	29-46

[a]Means in the same column followed by the same letters are not significantly different at the 5% level by Duncan's multiple range test.

largely the result of environmental factors and cultural practices affecting seedling development and ultimately, tip moth development.

The size of R. bushnelli reared in the trials was similar to the average size of R. bushnelli collected from P. ponderosa at Junction City in 1978 (Table 4).

Within each species and trial, mean forewing lengths of females were significantly larger than those of males. Size determination of field-collected moths also indicates females to be significantly larger than males.

With the exception of R. bushnelli reared on P. ponderosa in Trial II, mean development time for males was less than females within species. However, most of these differences were non-significant. In the field, emergence of adult males begins several days to a week earlier than female emergence (Dick 1969, Mortimer 1941).

Shoots infested with larvae varied in length and diameter. Therefore, in an attempt to obtain a more accurate index of larval consumption and correlate this with Rhyacionia size, dieback and shoot diameter measurements were multiplied for each tip moth reared. However, though it seems logical to assume that Rhyacionia consumption is positively correlated with size, when forewing length and adjusted dieback of individuals within each trial was plotted no such relationship was observed. From my observations, tip moth larvae may completely or only partially hollow out bud(s) and whereas some larvae may consume nearly all the tissue at the shoot tip, some prefer to feed primarily on the outer tissues for a greater length of the shoot. Thus, differences in preferred feeding areas may be responsible for the inability to correlate moth size with dieback.

Since rearing trials were conducted at different times in 1978, statistical comparisons between males and females from different trials were not made. The majority of shoot growth by _P. ponderosa_ and _P. sylvestris_ seedlings was complete before any rearing trials were conducted. However, mean shoot growth by uninfested _P. ponderosa_ in Trial I was 1.4 cm compared with an average increase of only .4 cm by seedlings in Trial II. Although males and females reared on _P. ponderosa_ in Trial I were slightly larger than those reared in Trial II, it is unclear whether _Rhyacionia_ size or development is affected by seasonal growth and development of the host.

In conclusion, the results obtained in the study indicate _R. frustrana_ and _R. bushnelli_ should not be considered separate species based on size.

Overwintering Behavior of Tip Moths in Kansas and Nebraska

Overwintering pupae in cocoons were found by sifting soil and debris collected under infested pines at Halsey and Cairo in Nebraska. Overwintering pupae were not found in soil at the Junction City, Kansas plantation, but a large number were found in the shoots. Several years ago, soil under infested pine at Hastings, Nebraska was examined by Nebraska forestry personnel (R. A. Read, personal communication 1977). No pupae were found in these samples which would indicate they probably overwinter in the shoots.

At Halsey, infested shoots examined in November 1977 and September 1978 did not contain pupae. However, four mid-instar larvae were found within the shoots in September. This might indicate that a partial third generation may occur in some years since pupation by the usual overwintering generation is normally completed by late August (Graham and Baumhofer 1927). However, these may be second generation larvae whose development had been impaired by food quality or parasitism.

At Cairo, it appears the majority of pupae overwinter in the soil. However, two live pupae were found in the shoots in both April and September in 1978. Under these circumstances, other evidence concerning reproductive isolation of the two forms is needed if the overwintering behavior criterion is to be considered a valid characteristic by which R. frustrana and R. bushnelli, if they are indeed distinct species, may be separated.

At present, the status of reproductive isolation is unknown. Morphological characters are not sufficiently different to indicate reproductive isolation. Physiological isolating factors such as the failure of zygotes to develop, or hybrid inviability or weakness, would be inefficient to the species energy resources. Behavioral differences are more likely involved. Since one species reportedly overwinters on the tree and the other in the soil, temperature differences could result in significantly different emergence periods for moths of the overwintering generation. However, the probability of overlapping flight periods would increase for the next generation. Sex pheromone specificity or differences in calling times by female moths would be very effective isolating mechanisms.

If populations which overwinter on the tree are not reproductively isolated from those that overwinter on the ground, they could be considered identical species or a semispecies (Mayr 1969).

Overwintering Potential of Kansas Tip Moths in Nebraska

Tip moths failed to emerge from infested shoots taken to Halsey in November of 1977 to determine if Kansas pupae could successfully overwinter on trees at this location. However, moths also failed to emerge from shoots collected in March 1978 at Junction City.

It is unclear why no adults emerged, especially from infested shoots collected at Junction City. Although spring emergence was approximately two to three weeks later than normal at the Junction City plantation, emergence was complete well before June 1,1978, when the contents of cages holding infested shoots were examined for moths. It is unlikely moths could have escaped from the cages since parasites of Rhyacionia, identified as Itoplectis conquisitor (Say), emerged in the cage containing shoots from Junction City. Itoplectis conquisitor does occur at Halsey (McKnight 1973), but it may either overwinter on the ground within tip moth pupae or utilize a different host. No parasites emerged from infested shoots taken to Halsey.

Examination of shoots returned from Halsey revealed 258 pupae, including 11 parasitized pupae in approximately 433 cm of infested shoots.

In March, when infested shoots from Junction City were collected and caged, additional shoots were obtained in order to remove pupae and determine mortality prior to emergence. From this collection, 270 pupae, including 15 parasitized pupae, were found in 564 cm of infested shoots. Of the 270 pupae, 219 appeared alive. From another collection, 133 pupae were obtained and placed in a petri dish and held in a refrigerator at a temperature of approximately 4 C. until June 1, 1978. Of these, 46 adults emerged in the laboratory within a period of several days.

In Kansas and southern Nebraska where tip moths overwinter as pupae within infested shoots, moth emergence begins in early April and continues for several weeks. The dates of emergence vary from year to year, depending upon weather conditions (Graham and Baumhofer 1927). At Halsey, adult emergence begins in early May and continues through the month (Swenk 1927).

At Junction City in 1978, adult emergence did not start until late April and was rather sporadic through the period because of unseasonably cold temperatures. At Halsey, however, the seasonal activity of tip moths was relatively normal. Since pupae overwinter in the soil at this location, this would indicate that spring emergence is dependent on soil temperature, not ambient temperature. It follows that the time of moth emergence at Halsey would be later than for tip moths which overwinter on the tree since those subjected to ambient temperatures would accumulate degree-days more quickly.

Temperature data presented in Tables 16 and 17 indicates ground and soil temperatures to be considerably warmer and more stable than ambient temperatures. Although these temperature data were recorded at Junction City, I assume soil and ground temperatures at Halsey would be very similar. Minimum ambient temperatures recorded at Halsey in January, February and March in 1978 are considerably colder than those for Junction City.

Based on the results obtained in this study, it remains unknown whether tip moth pupae can successfully overwinter on trees at Halsey. However, I suspect their ability to do so is largely dependent on the severity of winter conditions and that overwintering behavior of these moths in the Great Plains is temperature induced.

Table 16. Minimum temperatures (F.) occurring in the winter of 1977-78.

	December[a]		January		February		March	
	Min[b]	Mean	Min	Mean	Min	Mean	Min	Mean
Junction City								
Ground	20	27	13	21	13	25	-	-
Soil (½")	29	33	16	27	19	28	-	-
Soil (1")	32	34	18	27	19	28	-	-
Ambient	5	19	-7	9	-15	15	-9	25
Hastings	0	16	-14	2	-12	7	-12	23
Halsey	1	12	-26	-2	-23	1	-26	19

[a]Based on temperatures from December 16-31.

[b]Represents minimum temperature recorded during month.

Table 17. Maximum temperatures (F.) occurring in the winter of 1977-78.

	December[a]		January		February		March	
	Max[b]	Mean	Max	Mean	Max	Mean	Max	Mean
Junction City								
Ground	55	41	43	30	37	30	-	-
Soil (½")	49	37	33	31	34	31	-	-
Soil (1")	47	37	33	31	36	31	-	-
Ambient	68	45	52	28	48	30	80	50
Hastings	52	38	45	24	45	27	86	48
Halsey	52	37	50	26	48	28	85	50

[a]Based on temperatures from December 16-31.

[b]Represents maximum temperature recorded during month.

Response of Kansas Tip Moths to the Female Sex Pheromone of *Rhyacionia frustrana*

The results obtained from this study indicate that *Rhyacionia* males at Junction City, Kansas were strongly attracted to the female sex pheromone of *R. frustrana*.

Collection of traps baited with female pheromone on April 19 and 24, 1977 yielded 34 and 35 males, respectively, with no moths present in the control traps. No moths were collected from traps baited on May 2, however, the flight period of the overwintering generation is usually over by late April in northeast Kansas (Dick 1969). Thus it was concluded the first flight came to an end before May 2.

Where sex pheromones of Lepidoptera are not species specific, variations in adult morphology, mating rhythms, seasonal cycles, host plant selection and geographical distribution may influence the reproductive isolation of species (Roelofs and Comeau 1969). However, when closely related species are morphologically similar, sympatric in distribution and have similar ecological requirements, sex pheromones may be the primary factor responsible for maintaining distinct populations (Sanders 1971).

In the case of *R. frustrana* and *R. bushnelli* where no morphological differences are apparent, it seems likely sex pheromone specificity would be necessary to inhibit hybridization in areas where populations overlap.

One possible reproductive isolating mechanism not studied here involves the time period which females release the sex pheromone. Different calling times of the two species could prevent hybridization in the field.

SUMMARY

Problems in using size and overwintering behavior criteria to separate the Nantucket pine tip moth, Rhyacionia frustrana (Comstock) from the western pine tip moth, Rhyacionia bushnelli (Busck) are investigated.

Pupal weight and forewing length determinations were made for field-collected tip moths from Nebraska, Kansas, Oklahoma and Georgia. When compared by sex, tip moths collected from P. ponderosa in Kansas and Nebraska were considerably larger than those obtained from P. taeda in Oklahoma and Georgia. Tip moths at Halsey, Nebraska, which overwinter as pupae within cocoons in the soil, were no larger than tip moths at Hastings, Nebraska, which overwinter within shoots on the tree. However, pupae and adults collected from P. ponderosa at Junction City, Kansas were significantly smaller, when compared by sex, than those at Hastings. Also, tip moths at Junction City were significantly larger than moths which infest P. sylvestris Christmas trees at Manhattan.

In general, tip moth size may vary significantly with respect to location and generation. Generation within location differences are usually more pronounced for moths collected in

different years. Also, at each location sampled, females were significantly larger than males.

Factors which may influence tip moth development include larval density, environmental conditions, host species and the physiological state of the host. However, the exact nature by which these factors may affect tip moth size was not studied.

When compared by sex, no significant differences in mean forewing lengths were found between R. bushnelli and R. frustrana when reared on P. ponderosa or P. sylvestris seedlings in the growth chamber. Therefore, size should not be considered a valid criterion to separate the two species.

At Junction City, Kansas and Hastings, Nebraska, tip moths overwinter as pupae within the shoots. Pupae were found overwintering in cocoons at Halsey, Nebraska and Cairo, Nebraska, however, a few overwintering pupae were also found in the shoots at the latter location. This would indicate the existence of sympatric populations of these species if their present classification is correct, or a single species with variable overwintering habits.

An attempt to determine if Kansas tip moths can overwinter on trees at Halsey, Nebraska was not successful. However, their ability to do so probably is largely dependent on winter conditions. Furthermore, I believe that at each location where populations of these specie(s) occur there are some which overwinter on the ground and some which remain in the shoots.

The abundance of each form probably is dependent on winter temperatures.

Rhyacionia males at Junction City, Kansas were strongly attracted to the female sex pheromone of *R. frustrana*.

No one has investigated mechanisms other than morphological differences which may serve to reproductively isolate *R. bushnelli* and *R. frustrana*. If no such mechanisms exist, they could be considered a single species or a semispecies.

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Vita

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He received his B.S. degree from Kansas State University in the Entomology Science Option of the Crop Protection Curriculum in 1976. That same year, he began to study toward a Master's degree in the Department of Entomology. He was a Graduate Research Assistant under the supervision of Dr. Hugh E. Thompson.

On June 10 1978, he was united in marriage to Jane H. Archibald of Wichita, Kansas.

AN EXAMINATION OF SIZE AND OVERWINTERING BEHAVIOR CRITERIA USED FOR SEPARATING *RHYACIONIA FRUSTRANA* (COMSTOCK) FROM *RHYACIONIA BUSHNELLI* (BUSCK) (LEPIDOPTERA: TORTRICIDAE)

by

MELVIN V. HOLMAN

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Problems in using size and overwintering behavior criteria to distinguish the Nantucket pine tip moth, *Rhyacionia frustrana* (Comstock), from the western pine tip moth, *Rhyacionia bushnelli* (Busck) are investigated.

When compared by sex, no significant differences in mean forewing lengths were found between *R. bushnelli* and *R. frustrana* when reared on ponderosa pine, *Pinus ponderosa* var. *scopulorum* Englem. or Scotch, *P. sylvestris* L. seedlings under controlled conditions. Therefore, size should not be considered a valid criterion to separate the two species.

Pupal weight and forewing length determinations were made for field-collected tip moths from Nebraska, Kansas, Oklahoma and Georgia. Tip moths collected from *P. ponderosa* in Kansas and Nebraska were larger than those obtained from loblolly pine, *P. taeda* L. in Oklahoma and Georgia.

In general, tip moth size may vary significantly with respect to location and generation. Generation within location differences are usually more pronounced for moths collected in different years. Also, at each location sampled, females were significantly larger than males.

Factors which may influence tip moth development include larval density, environmental conditions, host species, and the physiological state of the host. However, the exact nature by which these factors may affect tip moth size was not studied.

Tip moth pupae were found overwintering within cocoons in the soil at Halsey, Nebraska and Cairo, Nebraska, however, a few overwintering pupae were also found in the shoots at the latter location. This discovery indicates the existence of sympatric populations of these species if the present taxonomic classification is correct.

An attempt to determine if Kansas tip moths can overwinter on trees at Halsey, Nebraska was not successful. However, their ability to do so is probably largely dependent on winter conditions. Furthermore, I believe that at each location where populations of these species occur, there are some which overwinter on the ground and some which remain in the shoots. The abundance of each form probably is dependent on winter temperatures

Rhyacionia males at Junction City, Kansas were strongly attracted to the female sex pheromone of *R. frustrana*.

Mechanisms other than morphological differences which may serve to reproductively isolate *R. bushnelli* and *R. frustrana* have not been investigated. If no such mechanisms exist, they could be considered a single species or a semispecies.