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### EFFECT OF GREEN VEGETATION AND COTTON NEST MATERIAL ON REPRODUCTION AND SURVIVAL OF PINE VOLES (MICROTUS PINETORUM).

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#### INTRODUCTION

Plant compounds associated with active plant growth and senesence have been reported to have different effects on microtine reproduction (Berger et al., 1977, 1981). Biological assays of inhibitory compounds showed some effect at pharmacological doses in Microtus pennsylvanicus but not in M. pinetorum (Cranford et al., 1980; Derting and Cranford, 1981). Stimulatory plant compounds contained in active growing wheat have been demonstrated to cause early sexual maturation (Berger et al., 1981) and to induce reproduction in wild populations during non reproductive periods (Negus, 1977; Berger et al., 1981). Bodenheimer (1949) postulated that plants contain compounds which trigger the onset of reproduction in M. guentheri. In M. montanus small amounts of green plants or their extracts supplemented to the normal laboratory diets have caused increased uterine weight, increased numbers of estrus females, increased numbers of young produced, and a return to sexual activity under normally inhibitory conditions (Pinter and Negus, 1965; Negus and Pinter, 1966; Negus and Berger, 1971; Negus and Berger, 1972).

Orchards typically have grass growing in aisle rows and under trees which could provide additional reproductive stimulation. Mowing keeps the grass in an active growth stage which would maintain a high level of reproductive stimulatory compounds in the vegetation. This research will report on the effect on reproduction of small amounts of oat sprouts fed to <u>M</u>. <u>pinetorum</u> in a laboratory colony and in animals maintained in large outdoor enclosures under natural conditions. An additional factor tested was the presence of nest material in the form of cotton batting.

#### MATERIALS AND METHODS

One hundred M. pinetorum were randomly selected at weaning from the laboratory colony and were paired at seventy days of age. All animals in the laboratory groups were maintained as mated pairs for 180 days under LD 16:8 in small tub cages with Wayne lab blox and water available ad libitum. Group one consisted of 10 experimental pairs which received the equivalent of 2 g dry weight of oat sprouts (greens) per day in addition to the normal diet and a control group of 10 pairs. Group two consisted of 10 experimental pairs which received cotton batting nest material in addition to the normal sawdust substrate and a control group of 5 pairs. Group three was composed of 30 animals, 10 males and 20 females, which were housed in outdoor enclosures as 10 groups of two females with one male. The outdoor enclosures were 45 cm by 180 cm in size and had 16 cm of earth for burrowing and subsurface nest construction. All groups had food and water ad libitum, were under natural photoperiod and temperature from June 1981 to December 1981, and had greens and natural vegetation

continuously available. Five of these groups (randomly selected) in addition to natural nest materials had cotton batting supplied as supplementary nest material.

All laboratory animals were weighed and examined periodically for signs of reproduction (scrotal testis, perforate vagina, enlarged nipples), litters were weaned at 21 days of age, sexed and recaged. Outdoor enclosure animals were checked daily and trapped periodically to determine the same parameters as the laboratory groups.

#### RESULTS

Laboratory group one was maintained on small supplemental feedings of greens and produced their first litters at  $69 \pm 7$  days of age while their control group was more variable and matured later (80  $\pm$  33 days). Overall litter size for the greens feed group was  $2.5 \pm 1$ while the control was smaller  $(2.1 \pm 9)$  and the sex ratio for greens fed animals was 52:48 while the control group produced more females (38:62). Over the 180 days of mated life, greens fed animals produced 3.57 litters per female at  $26.4 \pm 6$  day intervals while the control group produced fewer litters (2.43) and were more variable in the inter litter interval ( $40.9 \pm 12$  days). Both of these variables were statistically significantly different ( $\underline{P} = 0.025$ ;  $\underline{P} = 0.05$ respectively). Overall breeding success of the greens fed group was higher in every parameter including the mean number of offspring per female with the greens groups producing 8.86 young while controls produced 5.0 offspring per female.

Laboratory group two was maintained with supplemental cotton batting nest material resulting in 60 percent mortality over the test

period while only one control animal died. Reproductive success of supplemented cotton animals was 30 percent with most females only producing one litter. The point at which 50 percent of the females (LD 50) with cotton batting died was 56 days after the onset of the experiment with the male LD 50 at 100 days.

All animals in the group which survived the 180 day period were killed and all organs and tissues examined and weighed. No differences were noted between experimental (cotton present) and control animals except for mean body weights. Survivors which had cotton present were significantly heavier ( $\underline{P} = .01$ ) than the controls. For 12 animals which died, post mortum examination indicated that 7 died due to cotton blockage of the stomach-small intestine junction with 4 of the remaining 5 dying from cotton blockage of the small intestine-ceacum junction. The source of mortality of one control and one cotton animal could not be determined.

Group three housed in the outdoor enclosure under natural conditions with natural vegetation and greens present had 100% breeding success with 2.6 litters per female and a mean litter size of 2.43 in the absence of cotton. Three outdoor enclosures with cotton went extinct within 60 days producing no offspring, one enclosure survived and produced one litter and one enclosure group survived but did not breed. The post mortum examination of dead pine voles indicated that mortality was due to cotton blockage of the stomachsmall intestine junction. Initial population density in cotton containing enclosures was 15 which decreased to 9 while the same starting density in noncotton enclosures reached 46 at 135 days and

contained 2.6 breeding females per enclosure. Of the 46 individuals in the enclosures, the second, third and fourth litters produced over the 135 day period were clearly recognizable by pelage and or body size and weight. These cohorts were statistically different in body weight from each other and from the parental generation and their first litter (P < .05).

#### DISCUSSION

Although prior research with reproductive inhibitory compounds in M. pinetorum was not successful at biologically relevant dosage rates (Derting and Cranford, 1981) the effect of green vegetation even in small amounts was quite significant. Using the reproductive data from the indoor groups one can calculate the potential impact of grass (greens) present in the home range of a single female over a 120 day breeding period. Animals with grass would produce 21 offspring of which 10 would be females while animals lacking grass would produce 9 offspring of which 5.6 would be females. As has been shown by Negus et al. (1981) for M. montanus only very small amounts of the stimulatory compound 6-MBOA were necessary and our laboratory studies with oat sprouts indicate the same condition for M. pinetorum. Litter sizes for greens fed animals were higher both indoors and outdoors and the inter litter intervals were shorter. These effects have also been reported for M. montanus and our results are in close agreement with that data.

Cotton fiber present as supplementary nest material was injested by test animals and resulted in blocked segments of the stomach and small intestine. The effect of this blockage was to decrease the

animals digestive efficiency and resulted in decreased body weight and eventual death. The indoor experimental groups had lower reproductive success and those outdoors in the enclosure experienced nearly total mortality. The observed differences in time to death probably reflect differences in behavior of males and females. Females begin to die within two weeks of exposure but 50 percent died within 50 days while males took twice as long. This probably reflects the greater nest building and nest maintenance habits of the female.

The overall impact of greens and cotton in an orchard has yet to be tested but the outdoor enclosure experiment clearly indicates that the presence of grass in orchards probably enhances overall reproduction success. The presence of cotton in runways and adjacent to fossorial burrow entrances could perhaps contribute greatly to an overall management program. As cotton results in significant mortality, reduction in the numbers of litters produced and general reduction in individual nutritional status it could contribute to an overall management program. Because the voles must build nests and cotton is acceptable to the individuals their behavior alone could result in at least reduced recruitment into a population over time and perhaps extinction of females.

#### ACKNOWLEDGMENTS

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