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Effect of artificial treading on morphology and ethylene production in four herbage grasses

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ABSTRACT

The study was carried out in unheated glasshouse from May to December in 1994 in order to clarify the effect of treading on morphological responses, injured rate and ethylene production in four grasses (*L. perenne, S. bungeana, B. inermis and P. pratense*). Germinated seeds were planted in pots including river sand. After the 50th day, the treading treatments were subjected to the following three levels of vertical pressure on an aerial shoots 7 times at a 5-day interval : 0, 5 (middle) and 10 (heavy) kgf/cm^2.

Plant height was lower in treading treatments than in the control in four grasses. The position of shoot apex in a soil was lower in treading treatments than in the control in *L. perenne and B. inermis*, but the reverse result was obtained in *P. pratense* and *S. bungeana*. Total number of tillers and total DM weight were lower in treading treatments than in the control in four grasses. Injured rate was significantly higher in sheath than in leaf in four grasses. Injured rate was the lowest in *S. bungeana*, and the highest in *P. pratense*. Immediately after the first treading treatment, four grasses evolved high content of ethylene. In heavy treading treatment, ethylene production was 11.7 nl in B. inermis and 3.5-4.4 nl/plant/hour in other grasses. Ethylene production tended to decrease gradually on successive sampling days. It is suggested that morphological changes of grass species may be plastic adaptation against treading stress.

Key words : Artificial treading, Ethylene, Grasses, Injured rate.

Introduction

Pasture vegetation is maintained under various physical stresses such as treading, excreta deposition and aerial defoliation by grazing animals (Edmond, 1974). Specific plant species tolerant to these stresses are able to survive and continue to grow in pasture. Botanical composition of pastures is considered to be controlled by the rate of application of physical stresses. Few studies have been made on the effect of treading on herbage plant growth (Snaydon, 1981), although there are many studies on defoliation and excreta deposition (Watkins and Clements, 1978).

Mechanical stresses without any injury

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induce morphological changes in plants. The typical phenomenon is the depression of longitudinal elongation and the promotion of lateral growth (Hongo and Ohe, 1985; Jaffe, 1973). These morphological changes may be controlled by one or more plant hormones (Jaffe and Biro, 1979). Of many hormones, ethylene is said to play an important role in morphological changes (Goeschl *et al.*, 1966; Jaffe and Biro, 1979). The effect of animal treading on plant growth is said to be very complex, because treading stresses affect plant growth both directly by bruising or crushing and indirectly by compaction or puddling of soil (Edmond, 1974: Hongo and Oinuma, 1998). In sorghum, general ability of plants to tolerate stresses is reported to relate with electrolyte leakage, which is a good indicator for injured rate of tissues (Sultan and Ors, 1979).

The present study was designed to assess the effects of treading on morphological responses, ethylene production and injured rate of tissues in four herbage grasses.

Materials and methods

The experiment was conducted in an unheated greenhouse during 24th May to 1st December in 1994. The following four grasses were used: *Lolium perenne* L. (cv. Libel), *Stipa bungeana* TRIN., *Bromus inermis* LEYSS. (cv. Saratoga) and *Phleum pratense* L. (cv. Hokuo). The sand-culture method was used in this study to avoid soil compaction by treading. Used soil had previously been autoclaved for 60 min at 120 °C to sterilize soil fungi (Smith, 1973). Seeds were placed on 0.5 % agar medium in a petri dish for 7 days in an incubator at 22 °C. Three germinated seeds were planted in a stainless steel pot with 7-cm diameter and 4.5-cm height containing 180-g river sand. The sand was leached alternatively with distilled water and standard HOAGLAND's solution. The plants were grown for 43 days and three seedlings were thinned into one seedling with a similar size.

The treading treatments were subjected to the following three levels of vertical pressure on an aerial shoots, viz. 0 (control), 5 (middle) and 10 (heavy) kg/cm^2 with a hydraulic jack for 20 second. A pressure was applied on a concrete disk with 6-cm diameter, which was previously placed on a plant. Plants were trodden 7 times at a 5-day interval during 30 days. Three pots selected at random were arranged for each of three treatments.

Plants were sampled, washed and divided into leaf blade, sheath and root on the 80th day after sowing. Each organ was dried and its DM weight was measured. To measure the position of shoot apex, the mark was put on the sheath at a soil surface with an oil ink. Plants were harvested and the sheath was longitudinally sliced off with a razor blade (Edmond and Hoveland, 1972). Then, the length between the top of shoot apex and the mark was measured under a stereo microscope. An inclined angle of the secondary tiller was measured along a line formed by both a point of 5-cm height above soil surface and a basal point of tiller on a soil surface.

Produced ethylene and injured rate were measured immediately after each treading treatment. Then, plants were harvested 4 times at a 10-day interval during 30 days to measure injured rate. The analytical methods were the same as the previous report (Hongo and Oinuma, 1998).

Results

All plants of *P. pratense* and the half plant of *S. bungeana* were dead in the heavy treading treatment. The following results show the mean values of living plants in these treatments.

1. DM weight

Total DM weight was significantly lower in trodden plots than in the control (Fig. 1). The decreased rate of DM weight in trodden plots was the highest in *P. pratense* and the lowest in *B. inermis*. Root/shoot ratio of *S. bungeana* was

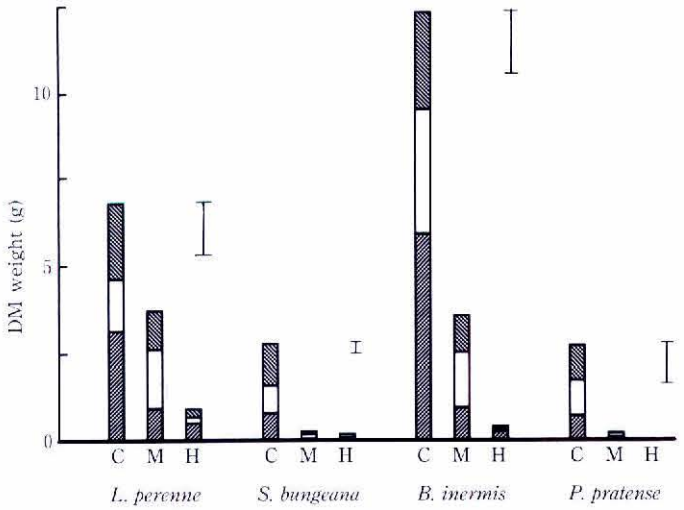

Fig. 1 DM weights of leaf, sheath and root in plots of the control (C), middle treading (M) and heavy treading (H) in four grasses. Vertical bars show S.E. ▨; root, □; sheath, and ▧; leaf.

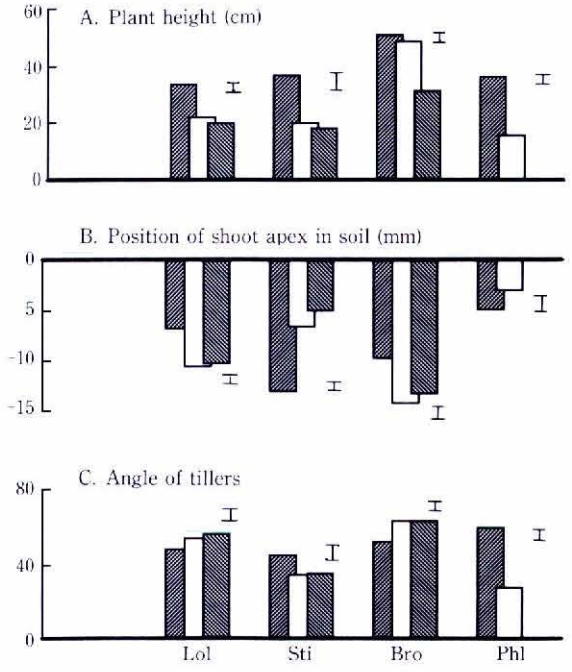

Fig. 2 Plant height, position shoot apex and angle of tillers in plots of the control (▨). middle treading (□) and heavy treading (▧) in four grasses. Vertieal bars show S.E.
Lol; *L. perenne*, Sti: *S. bungeana*, Bro; *B. inernis* and Phl; *P. pratense.*

significantly different from the other three species.

In *L. perenne*, moisture content of aerial shoots was 3.4 % lower in heavy treading treatment, compared with that in the control. There was no difference in other species.

2. Morphological response

Lower plant heights were observed in trodden plots in all four grasses (Fig. 2). The position of shoot apex was lower in trodden plots than in the control in *L. perenne* and *B. inermis*. The reverse tendency was observed in *S. bungeana* and *P. pratense*. In the latter species, young tillers emerged above dead aerial shoots, so that shoot apex located near the soil surface. The angle of the secondary tillers was different among four species. The secondary tillers in trodden plots emerged at an acute angle in *L. perenne* and *B. inermis*, but at an obtuse angle in *S. bungeana* and *P. pratense*.

Total number of tillers was lower in trodden plots than in the control in all four grasses (Fig. 3). Most of the primary tillers were dead in trodden plots in *S. bungeana*, *B. inermis* and *P. pratense*. The secondary and tertiary tillers vigorously emerged in *L. perenne* and *B. inermis*.

There was the significant correlation between plant height and total number of tillers. The correlation coefficient was $r=0.92$ in *S. bungeana* and $r=0.90$ in P. pratense. Plant height was positively correlated with the position of shoot apex ($r=0.93$) in *S. bungeana*. Total number of tillers was positively correlated with position of shoot apex in *P. pratense*.

3. Injured rate

With respect to injured rate, *S. bungeana* showed quite different response to treading. This species showed almost same values (4.2~6.8

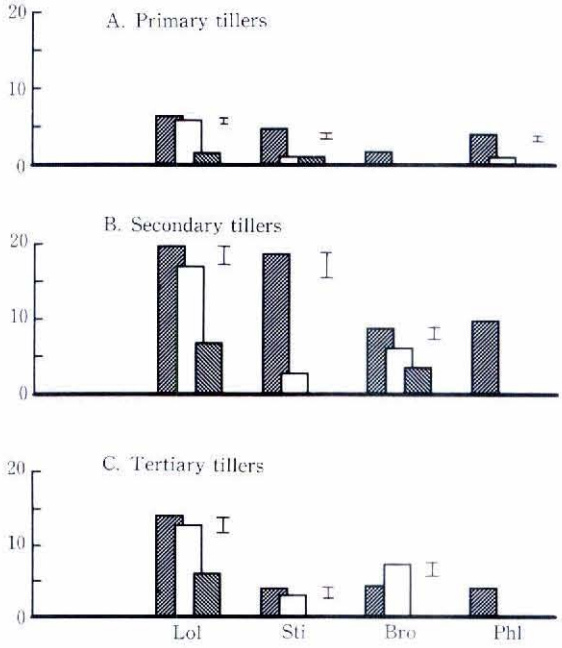

Fig. 3 Number of the primary, secondary and tertiary tillers in plots of the control (▨), middle treading (□) and heavy treading (▧) in four grasses. Vertical bars show S.E.
Lol ; *L. perenne*, Sti ; *S. bungeana*, Bro ; *B. inermis* and Phl ; *P. pratense*.

%) in both leaves and sheaths of all three treatments (Fig. 4). In other species, injured rates were significantly higher in sheaths than in leaves at four sampling dates. Injured rates of leaves and sheaths were about twice in heavy treading treatment than those in the control.

4. Ethylene production

Immediately after the first treading treatment, four grasses evolved high contents of ethylene. In the heavy treading treatment, produced ethylene was 11.7 nl/ plant/hr in *B. inermis*, 4.4 nl in *P. pratense*, 4.2 nl in *L. perenne* and 3.5 nl in *S. bungeana* (Fig. 5). These values were correspondent to 2.7, 1.4, 2.1 and 1.5 times, respectively, higher than the control. Ethylene production tended to decrease gradually on successive sampling days. As an average of two treading treatments, produced ethylene at the final measurement corresponded to 24, 23 and 75 % in *B. inermis*, *P. pratense* and *L. perenne*, respectively, compared with that at the first sampling. Produced ethylene was not significantly different between treatments in *L. perenne*, *S. bungeana* and *P. pratense*, because of wide variations.

Discussion

Morphology of four grasses was affected by repeated treading, resulting in a decrease in plant height and an increase in tiller number. The position of shoot apex in four grasses was also affected by repeated treading (Edmond, 1974). Through these morphological responses, it may be possible to preserve sensitive organs such as shoot apex, namely leaf primordium, from injury by treading stresses (Edmond and Hoveland, 1972). These responses seem to be a plastic adaptation in order to improve mechanical stability and physical strength (Hongo and Oinuma, 1998).

In this study, the degree of injury was esti-

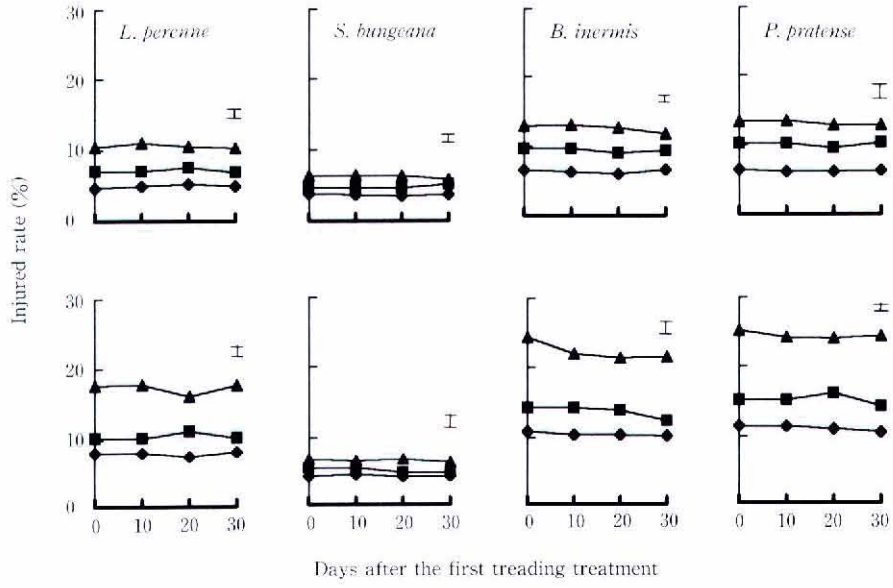

Fig. 4 Injured rates of leaf (upper figures) and sheath (lower figures) in plots of the control (◆), middle treading (■) and heavy treading (▲) in four grasses after the first treading treatment. Vertical bars show S.E.

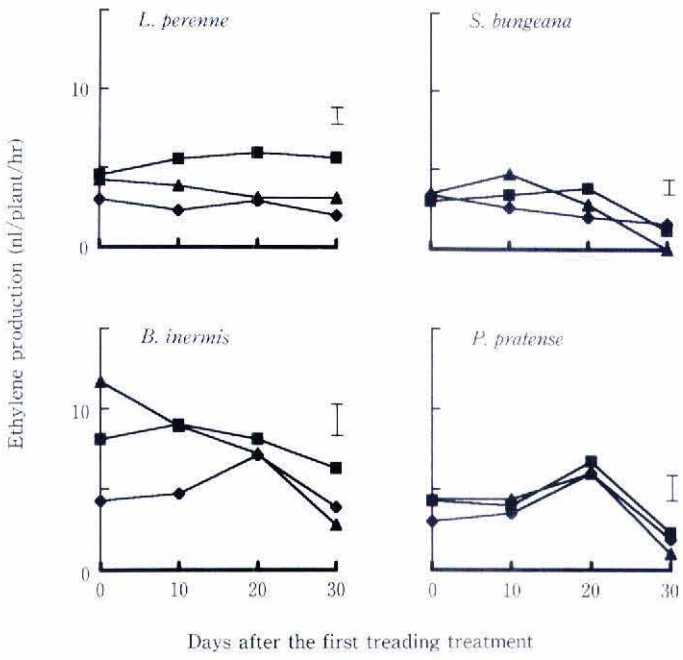

Fig. 5 Ethylene production in plots of the control (◆), middle treading (■) and beavy treading (▲) in four grasses after the first treading treatment. Vertical bars show S.E.

mated by the rate of electrolyte leakage. The treading treatments increased injured rate of leaf blade and sheath. In addition, injured rate was higher in sheath than in leaf. The higher rate of injury in sheath may be caused by its thickness. Usually, an external sheath includes 2–3 internal sheaths. Each sheath has almost the same thickness as that of one leaf. When treading stress is applied to grasses, piled up sheaths may receive more damage than leaf in a layer.

Mechanical stresses without injury for plants have been reported to induce morphological changes (Jaffe, 1973). Ethylene is said to play an important role in this morphological change, although other plant hormones also activate under various stresses (Jaffe and Biro, 1979). Ethylene production is proportional to logarithmic value of injured area (Imaseki *et al.*, 1968). In this study, ethylene production was significantly higher in trodden plots than the control in all four grasses. Furthermore, there was a significant correlation between ethylene production and injured rate of sheath in treading treatments. Thus, increased ethylene may relate with an area of injured organs. Sensitive organs, especially young sheath, may induce higher content of ethylene.

Under repeated treading, ethylene production tended to decrease gradually on successive sampling days, although injured rates were maintained at the same levels in each species. This result suggests that recovery rate of injured organs may be improved gradually by increasing hardness of cortical cell wall (Goeschle *et al.*, 1966). It is said that an increase in osmotic pressure also improves tolerance to mechanical stress (Abeles *et al.*, 1992). Drought resistance is also improved by mechanical stresses (Jaffe and Biro, 1979). In this study, a decrease in the moisture content of aerial shoots was observed

only in trodden plots of *L. perenne*. In this respect, further studies must be done.

Tolerance to treading stresses was different among four grasses according to DM production and morphological changes. *S. bungeana* and *P. pratense* may be classified into an intolerant group. *S. bungeana*, however, is a dominant species in overgrazed pastures in semi-arid areas of China. This means that the tolerant ability may change under different environments (Levitt, 1972). Then, further studies is necessary on the relationship between growth response of grasses and mechanical stresses under various kinds of environments.

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References

ABELES, F. B., P. W. MORGAN and M. E. SALTVEIT Jr. (1992) *Ethylene in Plant Biology*. Academic Press, San Diego.

EDMOND, D. B. (1974) Effects of sheep treading on measured pasture yield and physical condition of four soils. *N. Z. J. Exp. Agric.* **2**, 39-43.

EDMOND D. B. and C. S. HOVELAND (1972) A study of the position of growing points in two species under different systems of management. *N. Z. J. Agric. Res.* **15**, 7-18.

GOESCHL, J. D., L. RAPPAPORT and H. K. PRATT (1966) Ethylene as a factor regulating the growth of pea epicotyls subjected to physical stress. *Plant Physiol.* **41**, 877-884.

HONGO, A. and Y. OHE (1985) Effect of mechanical stress on growth of quackgrass (*Agropyron repens* (L.) Beauv.). *Weed Res., Japan.* **30**, 224-230.

HONGO, A. and H. OINUMA (1998) Effect of artificial treading on morphology and ethylene production in orchardgrass (*Dactylis glomerata* L.). *Grassld Sci.* **44**, 204-208.

IMASEKI, H., I. URITANI and M. A. STAHMANN (1968) Production of ethylene by injured sweet potato root tissue. *Plant and Cell Physiol.* **9**, 757-768.

JAFFE, M. J. (1973) Thigmomorphogenesis: the response of plant growth and development to mechanical stimulation. *Planta.* **114**, 143-157.

JAFFE, M. J. and R. BIRO (1979) Thigmomorphogenesis: the effect of mechanical perturbation on the growth of plants, with special reference to anatomical changes, the role of ethylene, and interaction with other environmental stresses. in *Stress Physiology in Crop Plants*. (Eds. H. MUSSELL and R. C. STAPLES) John Wiley and Sons, New York. pp. 25-59.

LEVITT, J. (1972) *Responses of Plants to Environmental Stresses*. Academic Press, New York. pp.544-568.

SMITH, A. M. (1973) Ethylene as a cause of soil fungistasis. *Nature.* **246**, 311-313.

SNAYDON, R. W. (1981) The ecology of grazed pastures. in *Grazing Animals* (Ed. F. H. W. MORLEY) Elsevier Sci. Pub. Com., Amsterdam. pp. 13-32.

Sultan, C. Y. and W.M. Ors (1979) Selecting for drought and heat resistance in grain sorghum. in *Stress Physiology in Crop Plants*. (Eds. H. MUSSELL and R. C. STAPLES) John Wiley and Sons, New York. pp. 263-301.

WATKIN, B. R. and R. J. CLEMENTS (1978) The effects of grazing animals on pastures. in *Plant Relations in Pastures* (Ed. J. R. WILSON) CSIRO, Melbourne, pp. 273-289.

イネ科牧草4種の形態とエチレン生成におよぼす人為的踏圧の影響

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要　　約

イネ科牧草の形態，組織の被害度，エチレン生成におよぼす人為的踏圧の影響を1994年5月～12月にガラス室において調査した。実験にはイネ科牧草4種（*Lolium perenne* L., *Stipa bungeana* TRIN., *Bromus inermis* LEYSS. and *Phleum pratense* L.）を用いた。実験には予め滅菌しておいた川砂を用いて，幼植物を砂耕栽培した。幼植物には蒸留水とホーグランド標準液を隔日に給与した。播種後50日目より，3段階（0，5，10 kgf／cm²）の踏圧処理を5日間隔で7回幼植物に加えた。

草高は対照区にくらべて踏圧処理区で低かった。*L. perenne*と*B. inermis*では土壌中の生長点の位置が踏圧処理区で低くなる傾向を認めたが，*P. pratense*と*S. bungeana*では逆の傾向を認めた。4草種とも，分けつの総数と総乾物重は対照区にくらべて踏圧区で低かった。電解質の溶出量から求めた組織の被害度は，4草種とも葉身よりも葉鞘で明らかに高かった。また，被害度は*S. bungeana*で最も低く，*P. pratense*で最も高かった。第1回目の踏圧処理直後において，*B. inermis*は11.7nl/個体/時間のエチレンを生成したが，他の3草種では3.5－4.4nl/個体/時間であった。これらのエチレン生成量は時間と共に減少していった。以上の結果から，踏圧ストレスに対するイネ科植物の形態の変化は可塑的な適応であることが示唆された。

キーワード：人為的踏圧，エチレン，イネ科植物，被害度