Non-destructive Method for Root Elongation Measurement in Soil Using Acoustic Emission Sensors

II. Spatial measurement of single root elongation

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Abstract: A non-destructive method for measurements of spatial root elongation in soil, using acoustic emission (AE) sensors, was developed. Growing roots passing in close proximity to soil particles generate AE pulses, which are detectable as counts by AE sensors. Previously, vertical maize (Zea mays L.) root elongation in soil was successfully measured using AE sensors. The method was expanded to measure spatial maize root elongation in a rectangular stainless steel container. Three AE sensors were placed vertically at 15 mm intervals on each of the four side walls of the container. Spatial root tip position was expressed by the three axes (x, y, z). Relative AE counts on the x axis and y axis (Rx and Ry) were obtained from three sensors on the same and opposite planes as a fraction of total AE counts on both sides of this plane. Rx and Ry were linearly related to root tip positions on the x axis and y axis, respectively. The relationship was expressed by the equation: Y = 0.740 - 0.0420X (r = 0.861)** where X is the actual distance from the sensor to root tip position and Y is Rx or Ry. Spatial root tip positions can be estimated by the relative AE counts using this regressing equation. Thus, spatial root tip positions were expressed as a function of time. The trajectory of root tip position estimated by AE sensors was consistent with that obtained from actual measurements after excavation of the root from soil. This method can be used to give continuous and three-dimensional information of root tip movements, the rate and direction of root elongation.

Key words: Acoustic emission, Circumnutation, Primary root, Root tip, 3-D analysis, Zea mays L.

Continuous measurement of root development is important for evaluating its contribution to water and nutrient uptake as well as its responses to changes in soil environment. Field methods such as the core sampling (Nakamoto, 1989) and monolith method (Morita and Okuda, 1995) are destructive and not suitable for the dynamic study of root development. Various non-destructive methods have been developed for studying the distribution of root systems in situ. In some of these methods, a transparent wall made of glass (Böhm, 1979) or other transparent material (Kaeriwama and Yamazaki, 1983) is placed against the soil profile or used as side walls of a specially constructed box filled with soil. Disadvantages of the transparent wall methods, however, are that some of the roots may be influenced by the soil-glass interface and may not reflect the actual root development under field conditions. In other methods, hydroponics (Tanimoto and Watanabe, 1986) is used to grow root without a soil matrix. The shape of the root system in hydroponics is different from that under field conditions. More sophisticated methods have been recently developed such as neutron radiography (Nakanishi, 1995) and NMR (Bottomley et al., 1993). These methods, however, need special instrumentation and are not suitable for continuous measurement of the root system.

A non-destructive method has been developed based on acoustic emission (AE). This method has been developed for the detection of cracks in structures. AE is "the phenomenon of the energy released, as sound pulses (AE pulses), from destroyed and/or deformed materials". AE pulses, generated by the elongation of pumpkin roots, have been used to detect root elongation (Okushima et al., 1996). Previously, we were able to detect AE pulses generated by elongating roots, and estimated continuously vertical root tip positions in the soil using AE pulses (Shimotashiro et al., 1998). This method was designed to examine only the vertical growth of a single root. Spatial measurements are required for the study of root tropism, such as gravitropism (Oyanagi et al., 1993), hydrotropism and electrotropism, and three-dimensional growth movements as a circumnutation. The final shape of a root system is determined by the expansion and direction of development of individual roots (Oyanagi et al., 1993). The objective of this study was to apply the AE method for spatial (three-dimensional) estimation of single root tip position in the soil.

Materials and Methods

1. AE measurement system

The AE measurements were made by using: (1) AE

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sensors (NF ELECTRONIC INSTRUMENTS, type NF AE 905WB, Yokohama kouhokku Tunajimahigashi 6-3-20, Japan) and (2) AE testers (NF ELECTRONIC INSTRUMENTS, type NF 9051s). AE sensors were connected to a data logger (Iwatsu SC8505) and a personal computer (Fujitsu FMV-7). AE testers were modified to eject a "high pass filter" and the frequency property was flat under 1 MHz. The AE counts were recorded if the pulse (detected signal) exceeded a preset lower level; this allowed elimination of unwanted background noise. Moreover, to exclude external electrical noise, AE counts were not recorded if the pulse exceeded a preset higher level. AE counts were recorded every ten minutes. More technical details were as described previously (Shimotashiro et al., 1998).

2. Simulation experiment using stainless steel rods

A container (15 × 15 × 200 mm) was constructed from stainless steel plates. Three AE sensors (12 mm in diameter) were vertically mounted on each of the four side planes at 15 mm intervals between their centers, which was the closest possible position (Fig. 1). The AE sensors on the stainless steel plate detect high frequency pulses. However, these pulses show rapid decay. Accordingly, the stainless steel plate shows no significant interference. The container was filled with sandy soil taken from Tottori Sand Dune, Japan. In this simulation experiment, the soil water content was 0% and soil bulk density was 1.5 g cm⁻³. It is suspected, however, that the difference of the soil might change due to the soil compaction caused by the filling procedure. A stainless steel rod (1.3 mm in diameter and 200 mm in length) was vertically pushed into the soil by a stepping motor at a rate of 1 mm h⁻¹ at four points on the x-y plane. These points in three dimensional coordinates (x, y, z) were (3, 7.5, 0), (6, 7.5, 0), (9, 7.5, 0) and (12, 7.5, 0) (mm).

3. Measurement of maize primary roots

The container was filled with sand and brought to a 20% water content with tap water. Pre-germinated maize (Zea mays L. cv. Snowdent) seed with 2 cm radicles were transplanted in the center of the container. The seedlings were grown in the dark at 27°C for three days. AE counts were recorded continuously. Then, the root was fixed spatially in the sand by an agarose gel and then excavated to take two photographs of root shape on the x-z plane and the y-z plane. The root shapes on the pictures were assumed to reflect the trajectory of root tip movements.

Results and Discussion

1. Simulation experiment using stainless steel rods

AE pulses were detected as AE counts in vertical order of the position of the sensors from top to bottom (Fig. 2). The transitional pattern of AE counts has one peak around the center of the sensor and was similar in all

![Fig. 1. Schematic diagram of root box arrangement and AE sensor position for rod simulation.](image-url)

![Fig. 2. Change of AE counts with rod tip positions on z axis, penetrated from four points on x axis. Vertical lines indicate center of AE sensor. X₁,₃ and X₂,₁ represent position on x axis in the two opposite planes. --- : Sensor X₀,₁, - - - : Sensor X₀,₁, - - - : Sensor X₁,₃, - - - : Sensor X₁,₁, - - - : Sensor X₂,₁, - - - : Sensor X₂,₃.](image-url)
sensors. The peak of AE counts at each penetration point was lower in the order of the number of sensors, 1, 2 and 3 from the top to the bottom, which may have resulted from the gradient in soil bulk density. Resistance to penetration increased with depth due to the increase in soil bulk density. Compared with the sensors at the same depth, where soil bulk density was the same, the level of AE counts were higher as the distance between the sensors and the rod penetrating positions on the x axis at the same depth was smaller. For instance, the peaks of AE counts detected by sensors X_{L3} and X_{R3} at the 3 mm penetration point were 1082 and 312, respectively. The peaks of AE counts detected by sensors X_{L3} and X_{R3} at the 6 mm penetration point were 823 and 388 respectively. These results suggested that the level of AE counts corresponded with the horizontal distance (on x axis and y axis) between the sensor and the rod tip. This relation was valid only when the rod penetration rate, soil bulk density and soil water content were constant. In the field, however, these parameters are variable. Estimation of rod tip position needs to be independent of these parameters. It was shown that AE counts increased with the rod penetration rate, soil bulk density and soil water content (Shimotashiro et al., 1998). The relative AE counts obtained from two vertically neighboring sensors, were calculated to standardize the AE counts (Shimotashiro et al., 1998). Thus, the relative AE counts on the z axis (Rzi) were calculated using the following equation:

$$\text{Rzi} = \frac{X_{zi}}{X_{L1} + X_{zi}}$$

where $X_{zi}$ is AE counts of the sensor on the $X_{zi}$ plane ($i$ is the sensor number) (Fig. 1). Rzi correlation with the distance to rod tip position on the z axis had the same slope and intercept as in the previous study (Shimotashiro et al., 1998). Relative AE counts on the x axis and y axis (Rx and Ry) were obtained from two horizontal sensors on the x axis and y axis. Rx and Ry were calculated by the following equations:

- $$\text{Rx} = \frac{X_{Xi}}{X_{L1} + X_{Xi}}$$
- $$\text{Ry} = \frac{Y_{Fi}}{Y_{L1} + Y_{Bi}}$$

where $X_{Xi}$, $Y_{Fi}$ and $Y_{Bi}$ are AE counts of the sensor on the $X_{Fi}$, $Y_{Fi}$ and $Y_{Bi}$ planes respectively ($i$ is the sensor number) (Fig. 1). Rx and Ry were calculated in the detection area from 10 to 65 mm obtained by three sensors. Changes of relative AE counts (Rx) obtained from two neighboring sensors in response to rod tip position on the z axis and at four points on the x axis are presented in Fig. 3. When the rod tip was near the sensor on the z axis, Rx values were close to those reported previously (Shimotashiro et al., 1998). When the rod tip position was far from the sensor, Rx tended to be variable at the same horizontal rod tip positions. This could be the reason why AE counts generated by the rod penetration were not large enough to be detected clearly and the ratio of signal to noise was low. It was assumed that Ry was the same as Rx because of their symmetry on the x axis and y axis.

For estimating spatial rod tip movements, the method of calculating relative AE counts was modified by using two opposite planes vertical to each of the three axis. X_{LP} and X_{RP} were obtained from the sum of the contributions of the three sensors on the same plane as follows:

![Fig. 3. Change of relative AE counts (Rx) obtained from neighboring two sensors in response to rod tip positions on z axis at four points on x axis. Vertical lines indicate center of AE sensor.

--- : Rx_{L1}, ---- : Rx_{L2}, -------- : Rx_{L3}.](image)

![Fig. 4. Change of relative AE counts (Rx_p) obtained from three sensors on one plane in response to rod tip positions on z axis at four points on x axis. Vertical lines indicate center of AE sensor.

--- : 3 mm penetration position on the x axis,

---- : 6 mm, ------ : 9 mm, --- : 12 mm.](image)
\[ X_{1p} = X_{1l} + X_{12} + X_{13} \]  
\[ X_{2p} = X_{2l} + X_{21} + X_{23} \]  
where \( X_{1l} \) and \( X_{2l} \) are the same as \( X_{1l} \) and \( X_{2l} \) in equation (2). Thus \( R_{xp} \) could be calculated by the following equation:

\[ R_{xp} = X_{lp}/(X_{1lp} + X_{2lp}) \]  

The values of \( R_{xp} \) were similar at each of the penetrating points on the \( x \) axis independent of the distance on the \( z \) axis (Fig. 4). \( R_{xp} \) at each penetrating point was linearly related to the distance between AE sensor center and rod tip position on the \( x \) axis (Fig. 5). The relationship was expressed by the following equation:

\[ Y = 1.02 - 0.0665X (r = 0.964^{**}) \]  
where \( X \) is the distance between rod tip position and the sensor, and \( Y \) was \( R_{xp} \). Root tip positions on \( x \) axis can be estimated using this equation independent of the distance on the \( z \) axis.

On the \( y \) axis, \( R_{yp} \) was calculated by the following equation:

\[ R_{yp} = Y_{fp}/(Y_{fpp} + Y_{bp}) \]  
where \( Y_{fp} \) and \( Y_{bp} \) are calculated in the same way as \( X_{lp} \) and \( X_{xp} \). The relationship between \( R_{yp} \) and the distance between AE sensor center and rod tip position, was plotted on the same regression line as in the case of \( R_{xp} \). The justification for this is the symmetry of rod tip positions on the \( x \) axis and \( y \) axis. Root tip positions on \( z \) axis can be estimated using equation (8) independent of distance on the \( z \) axis.

On the \( z \) axis, \( Z_{p-1} \) was the sum of four sensors \( (X_{1l}, X_{2l}, Y_{f}, \text{and } Y_{b}) \) positioned at the same depth. It was calculated by the following equation:

\[ Z_{p-1} = X_{1l} + X_{2l} + Y_{f} + Y_{b} \]  
where \( X_{1l}, X_{2l}, Y_{f}, \text{and } Y_{b} \) are the same as in equations (2) and (3). Therefore, relative AE counts \( (R_{zp-1}) \) on \( z \) axis was calculated by the following equation:

\[ R_{zp-1} = Z_{p-1}/(Z_{p-1} + Z_{p-1+1}) \]  

\( R_{zp-1} \) was linearly related to rod tip positions on the \( z \) axis. The relationship showed the same slope and intercept as in the simulation reported previously (Shimotashiro et al., 1998), and it can be estimated in the same way.

Consequently rod tip positions on each axis can be estimated by \( R_{xp}, R_{yp} \) and \( R_{zp-1} \) using these regression equations. This method can be adjusted for other types of soils by changing the slope and intercept in the regression equation.

2. Measurement of maize primary roots

AE pulses were detected during primary root elongation. \( R_{xp}, R_{yp} \) and \( R_{zp-1} \) were calculated from the same way as in the simulation. Actual root tip positions were determined by the excavated root shapes on the \( x-z \) and \( y-z \) planes, which were photographically recorded after the AE measurements. Root shapes were assumed to reflect the trajectories of root tip movements.

Estimated root tip positions on the \( z \) axis as a function of time was obtained from \( R_{zp-1} \) using the equation derived previously (Shimotashiro et al., 1998). Then, \( Rx \) and \( Ry \) were calculated and root trajectories were measured as actual root tip positions on the \( x \) axis and \( y \) axis respectively. There was no need to distinguish between \( Rx \) and \( Ry \) because of the horizontal symmetry on the \( x \) axis and \( y \) axis. Both \( R_{xp} \) and \( R_{yp} \) were significantly related to the distance from the actual root tip positions on \( x \) axis and \( y \) axis (Fig. 6). The distance from the actual root tip positions \( X \) on \( x \) axis and \( y \) axis was calculated from the following equation:

\[ Y = 0.740 - 0.0420X (r = 0.813^{**}) \]  
where \( Y \) is \( R_{xp} \) or \( R_{yp} \). Equation (11) was developed by regression of the \( R_{xp} \) and \( R_{yp} \) on the actual measurements of root tip position. The trajectory of maize root tip position estimated from relative AE counts was consistent with that obtained from the actual measurements (Fig. 7). As a result of this consistency, root tip positions estimated as a function of time could resemble the root tip movement. Furthermore, the rate (Fig. 8A) and


Fig. 7. Comparison between root tip trajectory determined by photographs of root morphology and by relative AE counts (\(R_{x_p}\) and \(R_{y_p}\)) in both the \(x-z\) plane and the \(x-z\) plane. The point 0 on the \(z\) axis represents the position at the start of measurement.

--- : root tip trajectory determined by final root morphology.

\(---\) : root tip trajectory determined by relative AE counts.

direction of root elongation (Fig. 8B) can be calculated from estimated root tip movement in the three dimensions.

The detected AE counts were recorded every ten minutes. The data were analyzed for the root tip movements, by summing up the AE counts every hour. The temporal resolution can be controlled by setting a data logger. A measurement time shorter than 10 minutes results in low signal/noise ratio, and makes the measured values unreliable. However, for root elongation measurement, as revealed by our study, the resolutions were satisfactory.

In this study, maize root elongation rate changed in the range of 0.5 to 4.0 mm h\(^{-1}\) and showed two peaks at 14 hours and 26 hours (Fig. 8A). These values are consistent with those of 1.3 and 3.1 mm h\(^{-1}\) reported by Yamazaki et al. (1983) and Sharp et al. (1988), respectively. Root elongation direction oscillated in the range of 0 to 60 degrees (Fig. 8B). The oscillation of root elongation direction is known as circumnutation (Spurry, 1974) but has not yet been investigated in soil. We were able to detect the circumnutation of the primary root in soil quantitatively and continuously. Root circumnutations are an important factor determining the elongation direction as well as root tropisms. In addition, seminal root circumnutations might cause morphological changes in the root system that improve root anchorage and plant establishments (Inoue et al., 1993).

Further research should address the limitation of the AE method which include: a) it is still limited to small containers since the AE sensor is capable of detecting root movement at distances of 15 mm or less, b) detection of elongation of more than one single root has not been solved and c) the method requires a uniform medium for root development.

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References


Nakanishi Y. 1995. Imaging of growing root and soil moisture around the root. Agriculture and Horticulture 70 : 62—68***.


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