GENERAL CONCLUSIONS

Results from the experiments reported in the preceding two manuscripts suggest that alaninyl-alanine (Ala-Ala), a biologically active dipeptide derived from corn gluten meal, is a potent inhibitor of rooting. The mechanism(s) by which this dipeptide exerts its activity is/are still unknown, however, the results from these experiments identify some possibilities.

The results from the first set of experiments described the treated root tips as being void of cellular components, specifically discernible nuclei and mitotic structures, with an overall loss of cytoplasmic integrity. Furthermore, treated root tips had extreme cell wall abnormalities including uneven thickening and breakage. Autoradiographs suggested that at the higher treatment concentrations, the epidermal tissue was adversely affected, thus decreasing the inward movement of the dipeptide. This could possibly be a result of abnormal cell wall thickening common to the epidermal cells in the treated roots. At the lower treatment concentrations, root tip necrosis was not evident, and inward movement of the dipeptide was not impeded.

In comparing the effects of this dipeptide with many of the commonly used synthetic herbicides, such as the dinitroanilines, carbamates, and dithiopyr, it was noted that many similarities between these root inhibiting compounds and the dipeptide exist. Review of the literature on mode of action studies for these synthetic herbicides provided a basis for further experimental approaches to elucidating the mode of action of this dipeptide. Common to much of this literature were studies to determine the herbicidal
effects on mitosis and cell ultrastructure of treated roots, thus leading to the objectives of the second part of this research.

Results from time-course experiments showed that Ala-Ala exhibited herbicidal activity within 4 h of exposure. By 6 h of exposure, reduction in the number of mitotic figures was nearly 100%, resulting in only interphase cells. At treatment concentrations where mitotic figures were present, none were aberrant and all four common mitotic stages were seen.

The combined results of this study lead to the conclusion that the detrimental effects of Ala-Ala on root meristem growth is the result of an inhibition of cell division rather than a disruption of cell division processes. Because only interphase cells were present and no absent or aberrant mitotic stages were noted, we conclude that Ala-Ala is acting on some metabolic process rather than directly on the mitotic apparatus. The observed effect on mitosis caused by Ala-Ala was different than the dinitroanilines, carbamates, and dithiopyr, all of which affect the mitotic apparatus (Vaughn and Lehnen, 1991), and was similar to the chloracetamides and sulfonylurea herbicides which affect plant metabolic processes (Deal and Hess, 1980; Rost, 1984).

The observed effects on cell walls and membranes, as well as the presence of lipid materials in vacuoles and intercellular spaces, implicates Ala-Ala as having membrane disrupting characteristics. Several classes of herbicides including the aryl-propanoic acids, cyclohexanediones, thiocarbamates, and chloracetamides have been reported to damage cellular membranes by affecting lipid synthesis (Devine et al., 1993). Whether or not
Ala-Ala causes a primary effect on lipid synthesis remains unknown and could be the focus of additional work.

**Literature Cited**


Cellular effects in perennial ryegrass (*Lolium perenne* L.) associated

with the root inhibiting compound alaninyl-alanine

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Corn gluten meal (CGM) has been shown to be an effective natural preemergence herbicide and fertilizer for various plant production systems. Alaninyl-alanine (Ala-Ala), along with four other dipeptides, were isolated from CGM and identified as being the inhibitory compounds. The herbicidal effects are seen as growth-regulating, root inhibitors that have minimal effect on shoots at low concentrations. Little is known about the inhibitory action of CGM or Ala-Ala.

The objective of the first phase of this research was to elucidate morphological and anatomical differences in perennial ryegrass seedlings treated with Ala-Ala using light and transmission electron microscopy, as well autoradiographic studies using $[^3]$H]-Ala-Ala. Results from these experiments described the treated root tips as being void of cellular components, specifically discernible nuclei and mitotic structures, with an overall loss of cytoplasmic integrity. Furthermore, root tips had extreme cell wall abnormalities including uneven thickening and breakage. Autoradiographs suggested that at high treatment concentrations causing epidermal tissue damage, there was minimal inward
movement of dipeptide. At lower concentrations, root tip epidermal necrosis was not evident, and inward movement of Ala-Ala was not impeded.

The objectives of the second phase of research were to use time-course studies to monitor the mitotic activity of roots treated with Ala-Ala; to use light and transmission electron microscopy to describe Ala-Ala induced changes in root cell ultrastructure; and to make comparisons with other reported modes of action of synthetic herbicides. Results showed that Ala-Ala exhibited activity on mitosis within 4 h of exposure, and by 6 h, reduction in the number of mitotic figures was nearly 100%, resulting in only interphase cells. Microscopic analysis revealed profound treatment effects. By 12 h, dense droplets, presumably membrane lipids, were visible in vacuoles and intercellular spaces. After a 48 h exposure, epidermal and cortical cell elongation in treated roots appeared to occur perpendicular to the normal elongation plane, possibly resulting from a loss of cell polarity. Root lateral branching was also noted after a 48 h exposure time.