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Morphology and anatomy of leafy spurge plants and tissue cultures: Interactions with herbicides

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Leafy spurge, *Euphorbia esula* or *E. podperae*, depending on the preference of names (1), is a perennial weed in pastures and other non-cultivated areas. Its control is a serious problem because it is spreading extensively and the cost of control is expensive partly due to the high cost of the chemicals used, and partly because those same chemicals are not translocated to the subterranean buds that are a major source of new plants. Morphological and anatomical studies of leafy spurge now in progress will serve as a basis for physiological studies aimed at chemical and biological control. This report is a preliminary survey of morphological features of seeds, leaves, and other structures of leafy spurge plants at various stages of growth. A scanning electron microscope (SEM) was used for detailed morphological observations. Freshly harvested leaves were coated with gold and palladium and examined directly without further processing. The leaves became dehydrated and distorted somewhat, but the wax structures and many stomata remained in good condition. Wax platelets on leaves and stems of greenhouse grown plants are about 0.5 to 1 μm (young leaves) and 1 to 3 μm (mature leaves) and appear to be similar on adaxial (upper) or abaxial (lower) leaf surfaces or on stems. The structures are also very similar between several biotypes grown in various parts of the United States and one from Austria. Wax structure on a field grown plant is similar to that on the same biotype grown in a greenhouse. One biotype (selected for physiological studies) was examined in greater detail than the others.

Seeds were also observed with a SEM. The raphe is a prominent feature that resembles a heavy log chain. Prominent pores occur near the raphe. The pores are roughly 15-20 μm in diameter and penetrate the seed surface about the same distance. Their function is unknown, but they trap fungal spores, and presumably bacteria. The hilum also is a structure that retains fungal spores.

Leaf replicas were made using red finger nail polish:acetone (1:1). The replicas were observed under a light microscope for stomatal patterns and to determine the numbers of stomata per unit area. The stomatal pattern of leaves from greenhouse-grown plants of this biotype varies according to the position of the leaf on the plant. Upper surfaces have stomata over the entire surface, but the numbers vary from about ten per mm^2 on the lower leaves to about 200 per mm^2 on the youngest leaves. The lower surfaces are more uniform than the upper surfaces, with about 150 to 200 stomata per mm^2 for all leaves.

However, the stomata are found only over the laminae on the lower surfaces and not over the midribs as on the upper surfaces. In general, the stomata appear to follow the pattern of the minor veins, being positioned directly over the veins. Stomata also occur on stems, but no detailed study of these structures was made.

Anatomical studies have been limited, but several prominent features were observed by means of light and transmission electron microscopy. Developing buds were fixed with buffered 2% glutaraldehyde followed by 1% osmium tetroxide, dehydrated and embedded in Spurr resin. Thick sections were stained with methylene blue-azure II for light microscopy. Thin sections were stained with lead and uranium and then were observed with a transmission electron microscope. Some light microscopy was done at low magnifications using hand sections of mature organs stained with coomassie brilliant blue, safranin, and several other stains. Laticifers were observed within the phloem tissues of developing buds and mature stems. Anatomical features in general appear to be those described by Myers *et al.* (2) except that the underground horizontal structures that contain the numerous buds do not appear to have a typical root anatomy (as claimed by Myers *et al.*) nor a typical stem anatomy. Other preliminary light microscopic studies have been done on the basic anatomy of leaves and developing root buds, using wax embedding techniques to obtain serial sectioned material of large structures.

Cell suspension cultures of eight leafy spurge biotypes are being maintained in our laboratory. The cultures grow well on at least two media that are used frequently for cell suspensions. One medium uses salts of Murashige and Skoog (3) and the growth regulators 2,4-dichlorophenoxyacetic acid (2,4-D) (0.4 mg/L), naphthaline acetic acid (0.4 mg/L, and kinetin (0.2 mg/L). Another medium designated as B5 (4) containing 0.1 to 1 µg/L 2,4-D, also works well and is used in most of our studies. These cultures are being used to determine the potential of several compounds derived from natural products and/or chemical synthesis as possible herbicides for leafy spurge control. The cultures are also being used to study basic physiology of organogenesis and plant growth regulation. Secondary cell wall formations have been observed in cells from cultures of all eight biotypes, and organogenesis has been observed in one biotype.

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