HISTOLOGICAL ANOMALIES IN STEMS OF COMMON AND RUNNER BEANS (*PHASEOLUS VULGARIS* L. AND *PH. COCCINEUS* L.) TREATED WITH PENDIMETHALIN

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ABSTRACT

Common beans (*Phaseolus vulgaris* L.) 'Augustynka' and 'Złota Saxa' and runner beans (*Ph. coccineus* L.) 'Blanka' and 'Eureka' were seeded on loess-like soil containing 1.6% of organic matter, and sprayed with pendimethalin at the dose of 1650 g·ha⁻¹ immediately after seeding in the middle of May. The herbicide inhibited shoot growth and caused enlargement of the stem at the soil level. Observations made in light and scanning electron microscope showed that in the swollen parts of the stem, the diameter of cortical parenchyma cells was bigger, the thickness of phloem layer was irregular, phloem fibers were less lignified, and the xylem cylinder was asymmetrical. In stems of 'Augustynka', 'Złota Saxa' and 'Eureka' cultivars, the thickness of secondary xylem and the diameter of vessels were reduced. Some vessels and tracheids were positioned transversely and obliquely to the stem axis and had an arched shape. Cell walls were less lignified and had a smaller number of pits. The largest number of histological anomalies was found in stems of the 'Augustynka' and 'Złota Saxa' cultivars.

KEY WORDS: pendimethalin, stem tissues, anatomical anomalies, *Phaseolus vulgaris* L., *Phaseolus coccineus* L.

INTRODUCTION

Pendimethalin is a selective soil-applied herbicide which has been used to control many annual grass and broad-leaved weeds in numerous crops for over 20 years. Germinating plants absorb pendimethalin through epidermis of roots, coleoptiles or young shoots which come into close contact with the herbicide (Herbicide Handbook 1994). Similarly to other dinitroanilines, pendimethalin inhibits the formation of mitotic apparatus microtubules (MTs) thus affecting chromosome movement and inducing formation of polyploid cells (Vaughn and Lehn 1991, Tarkowska et al. 1994). Moreover, Tarkowska et al. (1994) stated that pendimethalin acts on fragmoplast MTs causing formation of multinuclear cells and also on cortical MTs causing isodiametric growth of cells and disturbances in secondary cell wall structure. This leads to the formation of swellings on root tips (Beuret 1980, Smeda and Vaughn 1994) and at the base of a stem (Struckmeyer et al. 1976, Stalder et al. 1984, Borowy 1995). Bean stems injured this way become brittle, which makes mechanical harvesting of snap bean pods impossible (Stalder et al. 1984). Several authors have observed pendimethalin-caused injuries of bean plants in the course of field studies (Dobrzański et al. 1979, Trunkenboltz and Prin 1979, Haesaert et al. 1983, Stalder et al. 1984, Borowy 1995). However, reports on the anatomy of injured plant organs are scanty. In the pot

experiment carried out on common bean and soybean by Struckmeyer et al. (1976), cellular abnormalities in the swollen and brittle stem parts of plants treated with pendimethalin included: thinner cell walls, longer xylem elements, hypertrophy and hyperplasia of cells, and anomalous rings of vascular tissue in the stem cortical region. In Poland, two bean species: common bean (*Ph. vulgaris* L.) and runner bean (*Ph. coccineus* L.) are grown as vegetables. Common bean is cultivated both for mature seeds and for edible pods as snap beans while runner bean is cultivated only for mature seeds. Bean species and cultivars differ considerably in tolerance to herbicides (Borowy 1992, Haesaert et al. 1983, Lipska and Dobrzański 1978, Unterecker 1969). The purpose of this investigation was to determine the effects of pendimethalin on the anatomical stem structure of four bean cultivars belonging to two species cultivated in field and injured by the herbicide.

MATERIAL AND METHODS

Two cultivars of common bean (*Phaseolus vulgaris* L.): Augustynka grown for mature seeds and snap bean 'Złota Saxa' as well as two dwarf cultivars of runner bean (*Ph. coccineus* L.): 'Blanka' and 'Eureka' grown for mature seeds, were used for the presented investigation. A field experiment was carried out in the Experimental Farm in Felin on a loess-

like soil containing 1.6% of organic matter and of 12.3 mmol·100g⁻¹ cation exchange capacity. On May 12th 1994, 100 seeds of each cultivar were seeded 5 cm deep at 10 cm x 50 cm spacing on 8 experimental plots, each of 20 m². Immediately after seeding, 4 plots were sprayed with pendimethalin (Stomp, 330 g a.i. L⁻¹, Cyanamid) at the dose of 1650 g a.i. ha⁻¹ using a knapsack sprayer calibrated to deliver 400 L·ha⁻¹. The remaining 4 plots were considered as a control. Cumulative rainfall amounted to 52.3 mm after 3 weeks after seeding. A few days after germination, injury of bean plants caused by pendimethalin appeared. On June 16th, the height of all bean plants was measured and on July 13th six samples of swelled fragments of the base of stem of each cultivar were taken at the soil level randomly from different plots to perform anatomical studies. At that time runner bean plants were in the full blooming stage and common bean plants had first harvest suitable pods.

Light microscopy (LM)

Stem fragments were fixed in Carnoy liquid and then the transversal sections were made by hand and by means of Vibratome 2000 (Technical Product International, Inc., USA). Sections, 60 µm thick, were embedded in glycerine gelatine (Braune et al. 1975). Presence of lignin in cell walls was evaluated after treating the sections with phloroglucine and muriatic acid. Diameter of stem was measured. Number of cortical parenchyma layers was counted and thickness of phloem, xylem and secondary cortex was measured on stem sections in four opposite points. Moreover diameter of xylem vessels (80 vessels per treatment) in stems of both common bean cultivars, injured by pendimethalin in a greater degree than runner bean cultivars, was measured.

Scanning electron microscopy (SEM)

Fragments of stems were fixed in 4% glutaraldehyde with 0.1 M phosphate buffer of pH 7.2. Next they were fixed again in 1% osmium tetroxide and dehydrated in alcohol and 100% acetone. The samples were desiccated at the critical point with liquid CO₂ and coated with gold by means of CS 100 Sputter-Coater. Scanning microscope BS-Tesla was used for the observations. Transversal and longitudinal sections of stems were examined. The presence of pits in xylem vessels situated at the same distance to the stem axis was studied.

The data of measurements were analyzed by ANOVA and the Tukeys test was used for the statement of significance.

RESULTS

Shoot morphology

In both bean species, the shoot growth was reduced under the influence of pendimethalin (Table 1). The inhibition was most prominent in cultivars 'Blanka' and 'Złota Saxa'. Swelling of basal stem parts was observed in all cultivars. It was more distinct on common bean plants. The stem diameter of 'Złota Saxa' plants increased by about 6%, and that of Augustynka by about 13%, whereas in *Ph. coccineus*, the percentage of increases was 3% ('Eureka') and 4% ('Blanka') only.

Stem anatomy

Greater changes of internal structure after pendimethalin treatment occurred in stems of common beans. We observed them in the cortical parenchyma and in the layers of phloem and xylem (Figs 1-2).

TABLE 1. Plant height, stem diameter and thickness of cortical parenchyma, phloem and xylem layers and vessel diameter of the stem of control bean plants and of bean plants treated with pendimethalin.

Bean cultivars	Plant height [μm]		Stem diameter				Thickness of cortical parenchyma layer				Thickness of phloem layer				Thickness of xylem layer				Diameter of xylem vessel [μm]	
	Control	Pendimethalin	Control		Pendim.		Control		Pendim.		Control		Pendim.		Control		Pendim.		Control	Pendimethalin
			μm	%	μm	%	μm	% of diameter	μm	% of diameter	μm	% of diameter	μm	% of diameter	μm	% of diameter	μm	% of diameter		
Phaseolus vulgaris L.																				
'Augustynka'	93.0	82.0	6485.0	100.0	7306.0	100.0	382.4	11.8	517.8	14.2	225.3	6.9	180.8	4.9	845.7	26.1	745.4	20.4	92.0	75.3
LSD 0.05	10		336.6				67.8				32.3				n.s.				4.9	
'Złota Saxa'	95.0	70.0	6387.5	100.0	6743.7	100.0	361.7	11.3	437.4	13.0	175.1	5.5	181.8	5.4	1034.7	32.4	989.7	29.3	87.6	71.6
LSD 0.05	12.0		n.s.				51.3				n.s.				n.s.				4.9	
Phaseolus coccineus L.																				
'Blanka'	144.0	95.0	7005.0	100.0	7291.3	100.0	462.8	13.2	490.2	13.4	162.8	4.6	166.3	4.5	1128.6	32.2	1154.8	31.7	–	–
LSD 0.05	39.0		n.s.				n.s.				n.s.				n.s.				–	
'Eureka'	142.0	117.0	6739.5	100.0	6927.5	100.0	396.7	11.8	456.0	13.2	173.3	5.1	164.2	4.7	1079.6	32.0	955.3	27.6	–	–
LSD 0.05	n.s.		n.s.				58.6				n.s.				n.s.				–	

Figs 1-2. Fragments of cross sections of stem of *Ph. coccineus* 'Eureka'. Fig. 1. Stem of control plant. Fig. 2. Stem of plant treated with pendimethalin and showing disturbances in phloem and xylem organization. Xylem areas separated by un lignified tissue (arrows) are visible. C – cortical parenchyma, p – phloem, x – xylem, SEM.

The cortical parenchyma formed a considerably thicker layer in the presence of pendimethalin (Tab. 1). The number of cell layers of that tissue remained unchanged but the cell diameter increased by about 35% in 'Augustynka', 20% in 'Złota Saxa', 6% in 'Blanka' and 15% in 'Eureka' stems (comp. Figs 3 and 5).

Secondary phloem of control plants formed a ring of a uniform thickness. It was crossed by medullary rays of parenchyma and contained islets of phloem fibers in its peripheral part adjacent to primary phloem. The phloem layer in stems of plants treated with pendimethalin had variable thickness in both species. It was increased in some parts of a ring – reduced or approximating the phloem thickness of control plants (comp. Figs 1 and 2). These observations were confirmed by the measurements of secondary phloem thickness in stem sections (Table 1). Moreover, in stems of plants treated with pendimethalin, the bordering phloem fibers dis-

tinguished themselves by a very variable degree of wall lignification. The majority of fibers showed lignification only by the middle lamella or absolutely lacked lignin. Only few were lignified regularly.

Secondary xylem. In xylem of control plants, tracheal elements were regularly arranged in radial rows (Figs 1, 3, 8, 10). The thickness of the xylem cylinder surpassed the thickness of the peripheral parenchyma 2-3 times. Aggregations of xylem fibers and parenchyma cells alternated around the vessels (Fig. 11). The lignification of the cell walls of fibers and of tracheal elements was pronounced (Fig. 16). Under the influence of pendimethalin, the thickness of the xylem cylinder was reduced in the stems of 'Augustynka', 'Złota Saxa' and 'Eureka' cultivars, whereas in the stems of Blanka cultivar the reduction was much less prominent (Table 1). The diameter of xylem vessels in the stems of both species was smaller as well (Table 1, comp. Figs 1 and 2). In some stems,

Figs 3-7. Fragments of cross sections of stem of *Ph. vulgaris* 'Złota Saxa'. Fig. 3. Secondary structure of control plant stem with regularly arranged cells in xylem cylinder, x – xylem, p – phloem, c – cortical parenchyma. Fig. 4. Fragment of secondary xylem cylinder showing irregular structure after pendimethalin treatment: tracheal elements (t) separated with wide layers of unlignified tissue (n). Fig. 5. Cortical parenchyma showing increased cell size in stem treated with pendimethalin. Figs 6-7. Fragments of xylem in stem treated with pendimethalin. Tissue fragments showing irregularly arranged and unlignified cell walls (n) are visible. Some cells are elongated horizontally (arrow), LM.

Figs 8-9. Fragments of secondary xylem cross section in stem of *Ph. vulgaris* 'Złota Saxa'. Fig. 8. Xylem of control plant. Fig. 9. Xylem of plant treated with pendimethalin showing fragments of tracheal elements formed transversely to stem axis (arrows), SEM.

the xylem layer was asymmetrical and some of its fragments were thicker or thinner than the others (Fig. 2). The cell walls of xylem tracheal elements and of xylem fibers were less lignified and thinner in the majority of tested plants (Fig. 17). Cell walls of xylem fibers were easily deformed in the course of sectioning. However, the degree of this deformation was very variable. Xylem vessels and fibers of some plants treated with pendimethalin showed no abnormalities in wall lignification. In other plants, unlignified cells occurred in several parts of xylem cylinder (Figs 4, 6, 7). These cells were of a uni-

form size, undifferentiated to elements typical for xylem tissue and frequently not arranged in radial rows (Figs 6, 7). The arrangement of cells was disturbed in various xylem fragments. In its periphery the tracheal elements were situated as enclaves surrounded by parenchyma layers or occurred as undifferentiated and unlignified xylem cells (Fig. 4). The anomalies of the layout and of the shape of tracheal elements and xylem parenchyma caused by the herbicide were found also on cross and longitudinal stem sections. In stems of 'Złota Saxa', tracheal elements were laid out parallel but also

Figs 10-15. Xylem elements on cross section of 'Złota Saxa' stem. Fig. 10. Tracheal elements, fibers and parenchyma regularly arranged in radial rows (control plant), Fig. 11. Vessels (v) surrounded by fibers (f) and parenchyma (a) (control plant), Figs 12-14. Fragments of xylem after pendimethalin treatment. Tracheal elements elongated horizontally (h) and vertically (v) are visible, Fig. 15. Elongated living cells of xylem (l). M.

Figs 16-17. Xylem fibers and vessels on cross section of 'Złota Saxa' stem, Fig. 16. Xylem of control plant. Fig. 17. Xylem of plant treated with pendimethalin showing thinner-walled vessels and fibers (arrows), f – fibers, v – vessels. SEM.

transversely and oblique to the stem axis (Figs 9, 12-15, 18). Some of the xylem vascular elements had an irregular shape and some of them were arch-shaped (Fig. 19). Pits in this tissue were usually similar to those of control plants (Figs 20-23). However, they were less numerous (Fig. 21). The walls and several wall fragments of deformed tracheal elements in the stems of plants treated with pendimethalin did not have any pits (Figs 19, 23).

DISCUSSION

Pendimethalin induced changes in the anatomy of basal stem parts connected with increase of stem thickness. The anatomical abnormalities were more distinct in common bean cvs. This fact concurs with results of other authors showing the considerable sensitivity of this species to pendimethalin

and other dinitroaniline herbicides (Struckmeyer et al. 1976, Stalder et al. 1984, Borowy 1995). The abnormal distribution of xylem elements and the local lack of cell differentiation characteristic for this tissue were found in the different parts of xylem cylinder. This indicates that pendimethalin alters differentiation of xylem cells not only in the early stages of the plant development, but also in the later stages. Struckmeyer et al. (1976) described similar anomalies of the vascular tissue structure. The authors of the present study noticed that the organization of xylem vascular elements was changed due to the pendimethalin action. Some of the elements were horizontally and diagonally arranged and this made them longer on the transversal section than the cells of this tissue in control plants. It corresponds with the observations of Struckmeyer et al. (1976), who described the radial elongation of xylem elements.

Figs 18-19. Xylem fragments on radial longitudinal section of 'Złota Saxa' stem treated with pendimethalin and showing disturbances in tracheal elements (t) organization. SEM.

The present study showed that the diameter of tracheal xylem elements diminished, and the dimensions of cortical parenchyma cells increased under the influence of pendimethalin. However, Struckmeyer et al. (1976) reported the hypertrophy of the different types of cells within xylem and phloem in common bean stems treated with several dinitroaniline herbicides. The authors of the present investigation observed the thinner walled cells of considerable less lignification degree in lifeless xylem elements and in phloem fibers. This is probably one of the reasons of stem brittleness of common bean plants treated with pendimethalin during the field cultivation (Stalder et al. 1984, Borowy 1995). The decrease of the wall thickness within xylem cells was also described by Struckmeyer et al. (1976).

It seems that considerable deviations in the arrangement of xylem tracheal elements from their normal configuration, and the anomalies concerning the shape of vessels and tracheids may be the result of disorders of mitotic divisions caused by pendimethalin described by Beuret (1980). The abnormalities of that process probably concern also the changes of karyokinetic spindle orientation and disturbances of planes of the cell divisions. These changes could be caused by the action of pendimethalin on microtubules of mitotic spindle and fragmoplast and also on cortical microtubules responsible for the secondary cell wall structure, which was found by Tarkowska et al. (1994).

The obtained results show also that pendimethalin inhibited lignification of cell walls and development of pits.

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Figs 20-21. Vessel fragments on radial longitudinal section of xylem of 'Złota Saxa' stem. Fig. 20. Regularly situated pits in a vessel cell wall the control plant, Fig. 21. Vessel of plant treated with pendimethalin showing several fragments of cell walls devoid of pits. SEM.

Figs 22-23. Fragments of cell wall of xylem vessels in stem of *Ph. coccineus* 'Eureka'. Fig. 22. Vessel of control plant showing regular distribution of pits in cell wall, Fig. 23. Vessel of plant treated with pendimethalin showing lack of pits in the dark fragment of cell wall in the center of photograph, SEM.

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HISTOLOGICZNE ANOMALIE W ŁODYGACH FASOLI ZWYCZAJNEJ
(*PHASEOLUS VULGARIS* L.) I FASOLI WIELOKWIATOWEJ (*PHASEOLUS COCCINEUS* L.)
PO ZASTOSOWANIU PENDIMETALINY

STRESZCZENIE

Badano wpływ pendimetaliny na cechy anatomiczne łodygi dwóch gatunków: fasoli zwyczajnej (*Phaseolus vulgaris* L.) odm. 'Augustynka' i odm. 'Złota Saxa' oraz fasoli wielokwiatowej (*Phaseolus coccineus* L.) odm. 'Blanka' i odm. 'Eureka'.

Doświadczenie polowe założono na glebie płowej. Rośliny opryskano pendimetaliną w dawce 1650 g·ha⁻¹ bezpośrednio po siewie. W fazie kwitnienia mierzono długość pędów i pobrano próbki do badań anatomicznych. Przekroje podłużne i poprzeczne łodygi badano w mikroskopie świetlnym i skaningowym elektronowym.

Herbicyd spowodował zahamowanie wzrostu pędów oraz zwiększenie średnicy nasadowej części łodygi. W zgrubiałych łodygach stwierdzono powiększenie komórek parenchymy korowej, duże zróżnicowanie grubości warstwy floemu oraz asymetryczne formowanie cylindra ksylemu, którego grubość uległa redukcji. Zmniejszyła się również średnica elementów trachealnych, grubość ich ścian oraz stopień zdrewnienia.

Obserwowano zaburzenia w orientacji naczyń. Niektóre były położone poprzecznie lub ukośnie w stosunku do osi łodygi i miały łukowaty kształt. W ścianach elementów trachealnych obserwowano redukcję liczby jamek.

Największe anomalie histologiczne wystąpiły w łodygach odmiany 'Augustynka' i 'Złota Saxa'.

SŁOWA KLUCZOWE: pendimetalina, tkanki łodygi, anomalie anatomiczne, *Phaseolus vulgaris* L., *Phaseolus coccineus* L.