www.czasopisma.pan.pl PAN POLSKA AKADEMIA NAUK www.journals.pan.pl

JOURNAL OF PLANT PROTECTION RESEARCH Vol. 51, No. 4 (2011)

DOI: 10.2478/v10045-011-0074-0

PHYTOPHTHORA SHOOT BLIGHT OF PERIWINKLE IN POLISH HARDY ORNAMENTAL NURSERY STOCK

Leszek B. Orlikowski, Magdalena Ptaszek, Aleksandra Trzewik*

Institute of Horticulture
Konstytucji 3 Maja 1/3, Skierniewice, Poland

Received: April 24, 2011
Accepted: September 20, 2011

Abstract: In 2009–2010, *Phytophthora citrophthora* and *P. cryptogea* were consistently isolated from the diseased shoot parts of periwinkle (*Vinca minor* L.). The periwinkle had been collected from hardy ornamental nursery stock. The species were identified on the basis of colony patterns and their morphology as well as with molecular methods. Symptoms including yellowing and browning of leaves, dark brown or black spots enlarged on stems, and dieback of whole plants were observed. The symptoms were mainly noticed on cv. Aureovariegata and disease incidence was about 10%. Isolates of *P. citrophthora* and *P. cryptogea* colonized the leaf blades and stem parts of 7 periwinkle cultivars and 4 clones. Cultivars were colonized quicker than clones. Colonization trials showed that both species caused similar damage to periwinkle parts. The green clone of *V. minor* was more resistant to *Phytophthora* spp. than other colored clones. In a greenhouse trial, *P. citrophthora* and *P. cryptogea* inhibited rooting of periwinkle cuttings, especially Gertrude Jekyll and *V. minor.* This is believed to be the first report of *P. citrophthora* and *P. cryptogea* as the causal agents of *V. minor* shoot blight.

Key words: *Vinca minor*, *Phytophthora* spp., colonization, cultivars, clones

INTRODUCTION

Periwinkle (*Vinca minor* L.) is a popular plant grown in most Polish hardy ornamental nursery stocks. Plants are usually grown in 1 dm^3 pots and only sometimes in baskets. In such containers, 100 plants/m^2 are produced. Plants grow very densely, especially older ones. Until the last few years, the main problem in periwinkle growth was connected with the occurrence of Phoma shoot blight caused by *Phoma exigua* var. *inoxydabilis* Boerema and Vegh (Orlikowski 2001). Data obtained by some authors pointed to annual bedding, border and ground cover of Madagascar periwinkle [*Catharanthus roseus* (L.) G. Don.], known also as *Vinca rosea* L. (vinca), as the host plant of 5 *Phytophthora* species. The first information concerning Phytophthora blight caused by *P. parasitica* Dastur (= *P. nicotianae* var. *nicotianae* Breda de Haan) was published by Dastur (1916). In 1950, in Argentina, Frezzi noticed *P. citrophthora* (R.E. Smith & E.H. Smith) Leonian and *P. colocasiae* Raciborski on periwinkle as the causal agents of root rot, stem necrosis and foliar blight. Schubert and Leahy (1989) stated that only a few reports of the *Phytophthora* blight of periwinkle were found up until the early 1980's (Keim 1977), after which the disease severity increased in frequency. McMilian and Garofalo (2004) reported that in south Florida, *P. palmivora* (E.J. Butler) E.J. Butler, which occurred on the stems and leaves of commercial nursery potted periwinkle, was grown in the winter as a bedding plant. An outbreak of stem and root disease caused by *P. parasitica* was noticed in other large American nurseries. About 20% of infected plants with wilt symptoms showed extensive necrotic areas on stems and roots, rendering the plants unmarketable. The species can survive in the soil as chlamydospores or in plant debris, and there it can persist from season to season unless the soil is disinfected (Yandoc *et al.* 2007). Hao *et al.* (2010) observed foliar blight of annual vinca caused by *P. tropicalis* Aragaki and J.Y. Uchida in a large commercial nursery in Virginia. The disease began as dark, greenish-black lesions on young leaves. Lesions gradually became tan or brown and leaves wilted, curled and finally turned necrotic. Brown, sunken lesions, beginning at the branching points occurred on stems and shoots. Blighted areas on spots were 30 to 90 mm long and 20 to 40 mm wide on leaves, and 40 mm long on stems.

In the summer of 2009 and 2010, shoot blight symptoms were observed in one hardy ornamental nursery stock in the south-east part of Poland on *V. minor*. In the first year, disease symptoms were observed only in a few small places in the nursery. The next year about 10% of the plants showed shoot blight symptoms. The purpose of this study was (1) isolation, (2) identification of the causal agent of periwinkle blight and (3) to evaluate colonization of periwinkle cultivars by 2 *Phytophthora* isolates.

*Corresponding address:
Leszek.Orlikowski@insad.pl

MATERIALS AND METHODS

Plants

Observation was done on periwinkle grown in peat in 1 dm^3 pots. During the summer, plants were sprinkled with water as often as 3 times a day. Appearance of water-soaked and then grayish-brown and black lesions on the base of stems were noticed. Leaf color changed to yellow-green and light brown and then the leaves died (Fig. 1). Necrosis spread on shoots up to a length of 15 cm and also on leaf petioles and blades (Fig. 2). Invaded tissues were brown and on the ends, dark brown or black. During the next few weeks, dieback symptoms were observed on the most shoots and the disease spread to other plants (Fig. 3). Shoot blight symptoms were observed mainly on cv. Aureovariegata but also on other cultivars.

Fig. 1. Yellowing, browning and dying the of periwinkle affected by *Phytophthora* spp.

Fig. 2. Spread of necrosis on periwinkle shoots infected by *Phytophthora* spp.

Fig. 3. Plots of periwinkles in the nursery, affected by *Phytophthora* spp.

Sampling, detection of pathogens, and identification of *Phytophthora* spp.

The nursery was surveyed from the middle of July to the end of September, at 3 week intervals. Plants with disease symptoms were put together with their root system and substratum into plastic bags, and transferred to the laboratory. Invaded shoots were cut near the base of plants, washed under tap and deionized water, and dried between layer of sterile blotting paper. The isolation procedure described by Orlikowski and Szkuta (2001) was used for detection of *Phytophthora* spp. and other species. Diseased stem parts of 55 plants were disinfected over a burner flame and 3 mm long inocula were transferred on Potato Dextrose Agar (PDA) medium in 90 mm Petri dishes (12 inocula/dish). Within 48 hrs. of incubation at 24°C in the dark, possible *Phytophthora* colonies and other microorganisms were transferred on PDA slants. Isolates obtained were grouped by growth pattern and morphology into species "types". Representative colonies were then identified using the technique based on polymerase chain reaction (Trzewik *et al.* 2010). To confirm the identification, polymeraze chain reaction (PCR) with species – specific primers CRYF2/CRYR2 for *P. cryptogea* Pethybridge and Lafferty and Ctph1/Ctph2 for *P. citrophthora* was used (Èrsek *et al.* 1994; Boersma *et al.* 2000). The PCR reaction was performed in a Gene*Amp* PCR System (PE Applied Biosystems). The reaction mixture which was 25 µl in volume, contained 25 ng DNA, 0,5 U *Taq* polymerase (Fermentas), 0,4 µM of each primer, 50 µM of DNA nucleotide, and 1,5 mM $MgCl_2$. PCR parameters were as follows: 15 min of initial DNA denaturation at 95°C and 35 cycles of amplification (15 s of denaturation at 94°C, 60 sec of annealing at 64°C and 60°C for *P. citrophthora* and *P. cryptogea* respectively, 45 sec of elongation at 72°C), and 10 min of final elongation at 72°C.

Ten rhizosphere substratum samples, taken at different times from diseased plants, were also analyzed for the presence of *Phytophthora* spp. by soil baiting techniques using rhododendron leaves and methods described by Themann and Werres (1998) and Orlikowski and Ptaszek (2008). Samples of substratum were taken from separate containers with plants showing symptoms of wilting or yellowing and browning of shoots. Each sample of substratum was analysed separately. Leaves were laid in

10 mm of water standing on the surface of the flooded substratum taken from diseased periwinkles, and incubated at 22–24°C for 4 days. Leaf baits were removed, rinsed in tap and distilled water, and disinfected over a burner flame. Then, about 3 mm diam necrotic leaf parts were transferred on PDA medium. The rest of the procedure was similar to that done in the isolation of *Phytophthora* spp. from diseased stem parts.

Colonization of *V. minor* parts and cuttings by *Phytophthora* spp.

Seven cultivars and 4 types of periwinkle were used in the laboratory trials whereas three of them were used in greenhouse experiments. The procedure of Orlikowski and Szkuta (2001) was used for inoculation of leaf blades and stem parts in laboratory trials. Plant parts were put into trays with sterile blotting paper on the bottom, covered with nylon net. Isolates of *P. citrophthora* and *P. cryptogea* obtained from diseased stem parts were used for inoculation. Stock cultures were maintained for 7 days on PDA at 24°C, in the dark. Three mm diam mycelial plugs were transferred onto the base of stem parts and onto the middle of leaves. The control plant parts were inoculated with pure medium plugs. Trays were covered with thin foil and incubated at 22–24°C in the dark. After 7 days, the length and diameter of necrosis was measured.

In the greenhouse experiment, periwinkle cuttings were planted into 1 dm^3 pots with peat infested by *P. citrophthora* and *P. cryptogea,* using the procedure described by Orlikowski (1999). *Phytophthora* cultures for substratum infestation were grown on oats in 90 mm Petri dishes, sterilized twice at 120°C. After a 2 week incubation at 25°C in the dark oats overgrown by *Phytophthora* spp. were homogenized with 150 ml of distilled water in a Waring Blender to form a thick slurry. The homogenates were mixed with substrata at a rate one Petri dish per 1 l of substratum. The control cuttings were planted into noninfested peat. Pots were maintained on a bench covered with foil at temperatures ranging from 17° to 26°C. After a 2 week cultivation, the number of rooted cuttings and length of necrosis on the cuttings were evaluated.

The experimental design was completely randomized with 4 replications and 5 plant parts and cuttings in each replication. Trials were repeated twice at 2 week intervals. The results of the experiments were analyzed using analysis of variance (ANOVA). Significant treatment differences were evaluated by Duncan's multiple range test ($p = 0.05$).

RESULTS AND DISCUSSION

Isolation of *Phytophthora* spp. and other microorganisms from diseased plants and substratum

For 2 years, 55 periwinkles showing shoot rot symptoms were analysed. Ten genera and species were detected from diseased tissues with a domination of *Phytophthora* spp. (Table 1). Both, *P. citrophthora* and *P. cryptogea* were isolated with similar frequencies. Almost 90% of the analysed plants were settled by both species and they constituted about 50% of the obtained isolates (Table 1). *P. exigua* var. *inoxydabilis* was noticed only sporadically but *Botrytis cinerea* was detected from almost 13% of diseased plants, whereas *Fusarium avenaceum* from 9% of them (Table 1). Both *Phytophthora* species were also detected from 10 substratum samples. The species were noticed on at least 20 pieces of rhododendron leaf bait transferred on PDA medium in each Petri dish.

Colonization of periwinkle parts and cuttings by *P. citrophthora* and *P. cryptogea*

In the laboratory trials, both *Phytophthora* species colonized leaf blades and stem parts of periwinkle (Table 2). The quickest spread of necrosis was observed on leaves of cvs. Aureovariegata and Illumination (about 3 mm/24 hrs). On leaves of *V. minor* white-green and green, inoculated by *P. citrophthora,* necrosis developed significantly slower. The species did not colonize *V. minor* yellow-green (Table 2). On leaf blades of cv. Sebastian and 3 types inoculated by *P. cryptogea,* necrosis spread significantly slower than on other cultivars. Leaves of *V. minor* green were not colonized (Table 2). Both species colonized the

Table 1. Fungi and *Alge* like *Oomycetes* isolated from rotted shoots of 55 diseased plants of *V. minor*

Genera/species	Number of	
	colonized plants	isolates obtained
Acremonium sp.	3	5
Botrytis cinerea Pers.	7	12
Cladosporium herbarum Link	2	3
Fusarium avenaceum (Fr.) Sacc.	5	8
Mucor spp.	9	13
Penicillium spp.	11	19
Phoma exiqua var. *inoxydabilis* Boerema and Vegh	3	7
Phytophthora citrophthora (R.E. Smith & E.H. Smith) Leonian	28	45
Phytophthora cryptogea Pethybr. & Laff.	21	30
Trichoderma spp.	5	11

Table 2. Relationship between species and cultivars of *Vinca* spp. and colonization of plants parts by 2 *Phytophthora* species; length/diam of necrosis in mm, 7 days after inoculation, laboratory trial

Vinca minor cultivars/types	Leaf blades		Stem parts	
	P. citrophthora	*P. cryptogea*	*P. citrophthora*	*P. cryptogea*
Atropurpurea	15.5 ef	13.7 de	26.5 i–k	27.3 j–l
Aureovariegata	17.9 gh	18.2 h	22.7 f–h	30.4 l
Argenteovariegata	23.2 j	22.6 ij	23.4 f–i	22.3 f–h
Gertrude Jekyll	17.5 fg	15.9 fg	23.9 g–i	24.2 g–j
Illumination	23.3 j	20.8 i	25.0 h–k	27.8 kl
Ralph Shugert	16.7 f–h	15.6 ef	21.0 fg	20.0 f
Sebastian	16.8 f–h	11.7 cd	12.6 de	10.3 b–d
V. minor	12.2 cd	11.4 c	22.2 f–h	24.5 h–k
V. minor white-green	5.3 b	11.1 c	11.1 cd	15.2 e
V. minor green	5.6 b	0 a	7.1 ab	6.7 a
V. minor yellow-green	0 a	11.8 cd	7.9 a–c	10.0 b–d

Note: means in columns, followed by the same letter, do not differ significantly at the 5% level according to Duncan's multiple range test

Table 3. Relationship between *Phytophthora* species, *Vinca* cultivars and formation of roots on cuttings in the infested peat; mean number of cuttings* with first roots (n = 5)

Cultivars/clone of *V. minor*	*P. citrophthora*	*P. cryptogea*
Gertrude Jekyll	0.5 a	1.0 a
Sebastian	2.5 b	3.5 b
V. minor	0.8 a	0.8 a

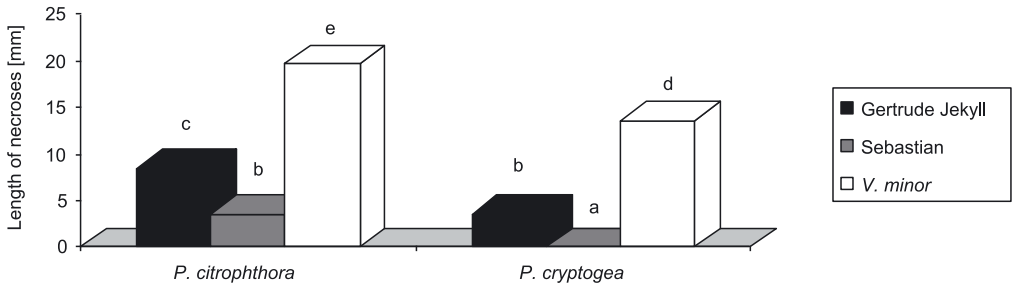
*mean number of rooted cuttings in noninfested peat = 4

Note: means followed by the same letter do not differ significantly at the 5% level according to Duncan's multiple range test

stem of all tested cultivars and types of *V. minor* (Table 2). *P. cryptogea* was a stronger colonizer of cv. Aureovariegata than *P. citrophthora*. Necrosis spread 4.3 and 3.3 mm/24 hrs, respectively. On other cultivars and types, there were no significant differences in the necrosis spread in relation to the *Phytophthora* species. Stem parts of cv. Sebastian and *V. minor* white-green, green and yellow-green, however, were colonized significantly slower (mean necrosis length 1.4 mm/24 hrs) than other cultivars (Table 2).

In greenhouse trials 4/5 of the cuttings rooted in noninfested peat (the control) produced roots (Table 3). Growing cuttings in peat infested with *P. citrophthora* and *P. cryptogea* resulted in the occurrence of stem rot on at least 4/5 of cv. Gertrude Jekyll and *V. minor*. On cv. Sebastian, separate from the *Phytophthora* species, at least half of the cuttings produced roots (Table 3). Observations of cuttings 14 days after planting, showed that necrosis had spread on 2 cultivars and 1 type of periwinkle in peat infested by *P. citrophthora,* and on Gertrude Jekyll and *V. minor* type in substratum with *P. cryptogea* (Fig. 4). The fastest colonization of tissues was observed on *V. minor* type (Fig. 4). To fulfill Koch's postulates, pathogens were reisolated from inoculated plants and diseased cuttings, and again identified.

In the survey on periwinkle, identification of obtained isolates implicated *P. citrophthora* and *P. cryptogea* as the major causes of shoot rot in the hardy nursery stock. Both species were isolated from most of the diseased plants of cv. Aureovariegata but not from other cultivars and types growing in the same nursery. This is believed to be the first report of both *Phytophthora* species attacking *V. minor*. Other isolated fungal species, except *P. exigua* var. *inoxydabilis*, probably colonized periwinkle shoots which were already infected by *Phytophthora* spp. The occurrence of *Phytophthora* spp. on only one cultivar, indicated the possibility of pathogens being brought in with young cuttings. Brasier (2008) indicated that "movement of plants and plant products between biogeographical zones by human activities is now generally accepted to be the primary mode of introduction of exotic pathogens and pests". The desire for novel species and ornamental plant cultivars by Polish nursery growers and landscape architects often resulted in the import of material from tropical and subtropical countries. During the last 20 years, 10 new *Phytophthora* species were detected from ornamental nursery plants (Orlikowski and Szkuta 2008; Orlikowski and Ptaszek 2010a, c). Among them was *P. citrophthora* isolated for the first time in the country, from *Pieris japonica* (Orlikowski and Szkuta 2001) and later from *Lavandula angustifolia, Syringa vulgaris, Forsythia intermedia* (Orlikowski and Ptaszek 2008; Orlikowski and Szkuta 2008), and from perennial plants (Ptaszek



Note: means on columns, followed by the same letter, do not differ significantly at the 5% level according to Duncan's multiple range test

Fig. 4. Relationship between *Phytophthora* species, *Vinca* cultivars and colonization of unrooted cuttings in the infested peat; length of necrosis (mm) 14 days after planting

and Orlikowski 2010). In the last 2 years, the species was detected from some coniferous plants and deciduous shrubs (Orlikowski and Ptaszek, unpubl.). *P. cryptogea* has been known in Poland since 1964, as the causal agent of gerbera wilt (Orlikowski 1978) but in the last 20 years it was detected from coniferous, deciduous and perennial plants (Orlikowski and Ptaszek 2007, 2008, 2010a, b, c). Periwinkle is the last discovered host of this species.

Both species are sometimes isolated from the same plants and are the causal agents of stem and leaf rot. Analyses of diseased periwinkle showed only necrosis of roots in the surface parts of the root system. The data obtained from laboratory trials indicated different reactions of cultivars and clones to *Phytophthora* spp. Periwinkle cultivars, in general, were colonized faster than clones, except *V. minor* inoculated by *P. citrophthora* and cv. Sebastian inoculated by both species. In greenhouse trials, that cultivar was much more able to resist severe root decay on *Phytophthora* spp. than Gertrude Jekyll and *V. minor* clone. In practice, under the conditions where plants are periodically sprinkled, severity of disease caused by both species is probably enhanced.

The presence of both species in substratum was connected with washing down of zoospores released from zoosporagia formed on the surface of diseased leaves and stems by sprinkling with water. Occurrence of shoot rot of periwinkle at even one place in the nursery, resulted in the spread of the pathogens to neighbouring plants, and dissemination of *Phytophthora* spp. to seemingly healthy landscape and garden plants. From such points, except in infected soil, the pathogens may settled locally growing plants. In spite of different reaction of periwinkle cultivars to Phytophthora shoot rot, consumer demand for them has been high and they will be widely grown in nurseries.

This work was financed by the Ministry of Science and Higher Education (Poland) under the project No. 475/N-COST/2009/0.

REFERENCES

Boersma J.G., Cooke D.E.L., Sivasithamparam K. 2000. A survey of wildflower farms in the south-west of Western Australia for *Phytophthora* spp. associated with root rot. Aust. J. Exp. Agric. 40: 1011–1019.

Brasier C.M. 2008. The biosecurity threat to the UK and global environment from international trade in plants. Plant Pathol. 57: 792–808.

Dastur J.F. 1916. *Phytophthora* on *Vinca rosea*. Mem. Dep. Agric. India, Bot. Ser. 8: 233–242.

Èrsek T., Schoelz J.E., English J.T. 1994. PCR amplification of species-specific DNA sequences can distinguish among *Phytophthora* species. Appl. Environ. Microbiol. 60: 2616–2621.

Frezzi M.J. 1950. Las especies de *Phytophthora* en la Argentina. Rev. Invest. Agric. Buenos Aires 4: 47–1 33.

Hao W., Richardson P.A., Hong C.X. 2010. Foliar blight of annual vinca (*Catharanthus roseus*) caused by *Phytophthora tropicalis* in Virginia. Plant Dis. 94, p. 274.

Keim R. 1977. Foliage blight of periwinkle in southern California. Plant Dis. Rep. 61: 182–184.

McMilian Jr. R.T., Garofalo J.F. 2004. *Phytophthora parasitica* wilt of new cultivars of *Catharanthus roseus*. Proc. Fla. State Hort. Sci. 117: 316–317.

Orlikowski L.B. 1978. The occurrence of *Phytophthora cryptogea* Pethybr. et Laff. in gerbera (*Gerbera jamesonii*) growing sites. Bull. Pol. Acad. Sci. 7: 495–498.

Orlikowski L.B. 1999. Selective medium for the evaluation of biocontrol agents efficacy in the control of soil-borne pathogens. Bull. Pol. Acad. Sci. 47 (2–4): 167–172.

Orlikowski L.B. 2001. Shoot blight of *Vinca minor* L. caused by *Phoma exigua* Desm. var. *inoxydabilis* Boerema et Vegh var. nov. J. Plant Protection Res. 41: 147–152.

Orlikowski L.B., Ptaszek M. 2007. *Phytophthora* spp. in Polish ornamental nurseries. I. Perennial plants, new hosts of *P. cryptogea*. J. Plant Protection Res. 47 (4): 401–408.

Orlikowski L.B., Ptaszek M. 2008. *Phytophthora cryptogea* and *P. citrophthora*; new pathogens of *Forsythia intermedia* in Polish ornamental hardy nursery stocks. J. Plant Protection Res. 48 (4): 495–501.

Orlikowski L.B., Ptaszek M. 2010a. Gatunki *Phytophthora* jako przyczyna zamierania żywotników (*Thuja* spp.) w polskich szkółkach kontenerowych. Sylwan 154 (4): 242–248.

Orlikowski L.B., Ptaszek M. 2010b. First notice of Phytophthora stem base rot on *Syringa vulgaris* in a Polish field nursery. J. Plant Protection Res. 50 (4): 442–445.

Orlikowski L.B., Ptaszek M. 2010c. Narastające problemy występowania chorób w pojemnikowej produkcji roślin ozdobnych. [Increasing problems of the disease occurrence in production of ornamental plants in containers]. Prog. Plant Protection/Post. Ochr. Roślin 50 (2): 678–686.

Orlikowski L.B., Szkuta G. 2001. Dieback of *Pieris japonica* caused by *Phytophthora citrophthora*. Acta Mycol. 36: 251–256.

Orlikowski L.B., Szkuta G. 2008. Zagrożenie szkółek pojemnikowych roślin ozdobnych przez *Phytophthora* spp. w minionym piętnastoleciu. Sylwan 9: 44–50.

Schubert T.S., Leahy R.M. 1989. Phytophthora blight of *Catharanthus roseus*. Plant Pathol. Circ. 321, 2 pp.

Ptaszek M., Orlikowski L.B. 2010. Zagrożenie niektórych roślin w szkółkach pojemnikowych przez gatunki *Phytophthora* wyizolowane z bylin. Zesz. Probl. Pos. Nauk Rol. 554: 189–194.

Themann K., Werres S. 1998. Vervendung von Rhododendron blattern zum Nachweis von *Phytophthora*-Arten in Wurzeln- und Bodenproben. Nachr. Dtsch. Pflanzenschutz. 50: 37–45.

Trzewik A., Ptaszek M., Orlikowska T., Orlikowski L.B. 2010. Wykorzystanie techniki PCR w identyfikacji *Phytophthora* do gatunku [Identification of *Phytophthora* species using PCR technique]. Prog. Plant Protection/Post. Ochr. Roślin 50: 756–759.

Yandoc C.B., Rosskopf E.N., Shah D.A., Albano J.P. 2007. Effect of fertilization and biopesticides on the infection of *Catharanthus roseus* by *Phytophthora nicotianae*. Plant Dis. 91: 1477–1483.