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Plant Diseases and Diagnosis

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Phytophthora chrysanthemi
(Naher, Hi. Watan., Chikuo & Kageyama)

Julius Kühn-Institut, Federal Research Centre for Cultivated Plants

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History

In 1998 a root rot on *Chrysanthemum* caused by an unknown *Phytophthora* species was observed in Japan. In 2003 the pathogen was detected again in Japan, in hydroponically grown potted chrysanthemum (Watanabe et al. 2007). This unknown *Phytophthora* species was identified as *Phytophthora chrysanthemi* sp. nov. (Naher et al. 2011).

In Europe *P. chrysanthemi* was first detected in Croatia in 2008 and 2009 (Tomić and Ivić 2015) on *Chrysanthemum* cultivated in a nursery. In 2015 *P. chrysanthemi* was detected in a German ornamental nursery on *Chrysanthemum indicum* (Götz et al. 2017). A further *Phytophthora* isolate detected in a German ornamental nursery in 2016 was also identified as *P. chrysanthemi* (Götz, Ulrich, Werres, unpublished data).

Recently *P. chrysanthemi* was detected in the United States on *Chrysanthemum* x *morifolium* 'Aubrey Orange' and 'Fancy Ursula Orange' (Lin et al. 2017).

Geographical distribution

Asia: Japan (Naher et al. 2011)

North America: USA (Lin et al. 2017)

Europe: Croatia (Tomić and Ivić 2015)
Germany (Götz et al. 2017)

Host range

Chrysanthemum sp.

Chrysanthemum indicum

Chrysanthemum x *morifolium* 'Aubrey Gold', 'Aubrey Orange', 'Fancy Ursula Orange'

Disease symptoms

See JKI Data Sheet '*Phytophthora* on *Chrysanthemum* (chrysants, mums)'

Biology / Morphology / Sequence Analysis

- *P. chrysanthemi* belongs to clade 9/10, a clade with many high-temperature tolerant *Phytophthora* species.
- The species can produce chlamydospores and oospores.
  It is a homothallic *Phytophthora* species.

(Fig. 1, 2, 3)

Tab. 1: *Phytophthora chrysanthemi* isolates

Isolate no. Original	Subcultures	Country of sample origin	Year of isolation
GF749	CBS123163 (ex-type)	Japan	2003
Chr3	JKI-026-16-8-00-0-0 [1]	Japan	1998
GF749	JKI-027-16-8-00-0-0 [1]	Japan	2003
PH_47	-	Croatia	2008
JKI-050-15-8-01-2-0	CBS 142199	Germany	2015
JKI-050-16-8-01-2-0	-	Germany	2016
201600114 ODA	114 JKI-009-17-8-00-0-0 [2]	USA (Ohio)	2016
201500542 OSU	542 JKI-010-17-8-00-0-0 [2]	USA (Ohio)	2015
FPH2016-102/201500543 OSU	FPH2016-102 543 JKI-011-17-8-00-0-0 [2]	USA (Ohio)	2015

The isolates were kindly supplied by [1]Koji Kageyama, Gifu University, Japan; [2]Paul Tooley, USDA-ARS, USA

Tab. 2: Morphological characteristics of *Phytophthora chrysanthemi*
2.1 Vegetative growth

Isolates studied	Agar medium	Vegetative growth Minimum	Optimum	Maximum	growth rate at optimum temperature (mm/24h)	References
		temperature (°C)				
hr3, GF749	V8	5	20	35	4.1	Naher et al. 2011
JKI-026-16-8-00-0-0	CPA	10	28	32	4.3	Werres, unpublished data
JKI-027-16-8-00-0-0	CPA	10	25-28	35	3.5	
PH_47	CPA	N/A	30	35	N/A	Tomić and Ivić 2015
JKI-050-15-8-01-2-0	CPA	10	30	35	4.1	Götz et al. 2017
JKI-050-16-8-01-2-0	CPA	10	25	35	3.1	Werres, unpublished data
JKI-009-17-8-00-0-0	CPA	10	28-32	35	3.2	Werres, unpublished data
JKI-010-17-8-00-0-0	CPA	10	30-32	35	3.4	Werres, unpublished data
FPH2016-102	LBA	15	28-30	32	1.5	Lin et al. 2017
JKI-011-17-8-00-0-0	CPA	15	30	32	1.5	Werres, unpublished data

V8 = Vegetable juice agar, V8+$CaCO_3$ = Vegetable Juice agar+$CaCO_3$, CPA = Carrot Piece agar,
ChryA = *Chrysanthemum* agar, LBA = Lima Bean agar, N/A = no data available

2.2 Sporangia and chlamydospores

Isolates studied	Sporangia					Chlamydospores			References
	n	L x B		L:B ratio		n	range (µm)	average (µm)	
		range (µm)	average (µm)	range	average				
Chr3, GF749	N/A [1]	24-59 x 16-43	42.2 x 27.9	N/A	1.4	N/A[1]	27.0-46.0	39.3	Naher et al. 2011
JKI-026-16-8-00-0-0	only very low numbers developed [2]					50 [2]	26.2-50.0	35.7	Werres, unpublished data
JKI-027-16-8-00-0-0	only very low numbers developed [2]					50 [2]	21.8-41.6	30.9	Werres, unpublished data
PH_47	50 [3]	22-61 x 18-45	N/A	N/A	N/A	50 [2]	25 - 50	N/A	Tomić and Ivić 2015
JKI-050-15-8-01-2-0	7 [2]	35.4-63.1 x 20.4-39.2	53.7 x 31.6	1.51-2.04	1.73	50 [2]	31.1-56.9	44.0	Götz et al. 2017
JKI-050-16-8-01-2-0	50 [3]	34.2-54.2 x 23.3-37.3	44.3 x 30.0	1.23-1.99	1.51	50 [2]	21.1-43.3	31.5	Werres, unpublished data
JKI-009-17-8-00-0-0	none developed [2]					50 [2]	30.0-46.0	37.8	Werres, unpublished data
JKI-010-17-8-00-0-0	only very low numbers developed [2]					50 [2]	26.6-45.9	34.6	Werres, unpublished data
FPH2016-102	13 [4]	26.6-41.0 x 21.5-25.35	N/A	N/A	N/A	N/A [4]	N/A	N/A	Lin et al. 2017
JKI-011-17-8-00-0-0	none developed [1]					50 [1]	25.8-55.7	41.1	Werres, unpublished data

N/A = no information available, [1] on V8, [2] on CPA, [3] on V8+$CaCO_3$, [4] on LBA (abbreviations see table 2.1)

Shape of Sporangia	Chlamydospores / Hyphal swellings	References
mainly terminal, noncaducous, nonpapillate, ellipsoidovoid or pyriform	chlamydospores: terminal, lateral or intercalary, and sometimes abundant on mycelium hyphal swellings: mostly lateral, intercalary, elongated, irregular to globose	Naher et al. 2011
nonpapilate, noncaducous, obpyriform, ovoid, terminal on sporangiophore, rarely sympodial, often with internal extended proliferation	chlamydospores: mainly intercalary and terminal, spherical hyphal swellings: mainly lateral, intercalary less often terminal, spherical to elongated	Tomić and Ivić 2015
nonepapillate, empty sporangia have a very wide exit pore (<12 µm), ovoid-ovate, ovoid-limoniform/pyriform, wide, noncaducous, sporangiphores: mainly simple sympodal	chlamydospores: terminal and intercalary, single and in groups hyphal swellings: elongated, irregular to globose	Götz et al 2017 Werres, unpublished data
unbranched, nonpapillate, nonecadocous, pyriform, ovoid or ellipsoid	N/A	Lin et al. 2017

N/A = no information available

2.3 Oogonia, antheridia and oospores

Isolates studied	Oogonia			Antheridia				Oospores			References
	n	range (diameter, µm)	average (diameter, µm)	n	L (µm)	B (µm)	L x B (average, µm)	n	range (diameter, µm)	average (diameter, µm)	
N/A	N/A [1]	26-46	38.6±3.4	N/A [1]	12-24	7-19	19±5.9 x 12.1±3.9	N/A [1]	19-35	29.4±3.98	Naher et al. 2011
JKI-026-16-8-00-0-0	25 [2*]	26.0-44.1	37.8	22	9.7-25.3	11.3-18.5	17.4 x 15.0	24 [2]	22.1-36.9	31.6	Werres, unpubl. data
JKI-027-16-8-00-0-0	22 [2*]	34.0-44.9	39.5	10 [2]	10.9-19.8	10.0-21.3	16.0 x 13.2	22 [2]	27.8-40.2	32.8	Werres, unpubl. data
PH_47	50 [2]	25-49	N/A	50 [2]	10-25	8-18	N/A	50 [2]	22-38	N/A	Tomić and Ivić 2015
JKI-050-15-8-01-2-0	50 [3]	27.1-45.2	35.4	43 [3]	9.9-21.2	9.2-19.4	15.6 x 13.5	50 [3]	21.3-35.4	28.4	Götz et al. 2017
JKI-050-16-8-01-2-0	only very low numbers developed [2,3]										Werres, unpubl. data
JKI-009-17-8-00-0-0	50 [2]	33.3-46.1	38.1	38 [2]	10.6-25.6	5.6-17.0	17.0 x 11.8	50 [2]	27.6-39.6	33.0	Werres, unpubl. data
JKI-010-17-8-00-0-0	50 [2]	22.2-44.4	35.8	45 [2]	9.4-22.7	8.6-16.2	16.4 x 12.3	50 [2]	18.7-38.2	30.5	Werres, unpubl. data
FPH2016-102	-	N/A	N/A	-	N/A	N/A	N/A	15 [4]	18.91-24.98	18.47-25.78	Lin et al. 2017
JKI-011-17-8-00-0-0	32 [2]	27.5-41.8	33.2	21 [2]	12.4-22.4	9.4-16.1	17.6 x 12.7	32 [2]	22.4-37.8	29.9	Werres, unpubl. data

N/A = no information available; [*] many oospores are degenerated and developed in clumps; [1] on V8, [2] on CPA, [3] on ChryA, [4] on LBA (abbreviations see table 2.1)

Shape of Oogonia	Antheridia	Oospores	Reference
terminal, often laterally sessile, intercalary, mostly spherical or nearly spherical, occasionally possessing a distinct funnel-shaped stalk	terminal, occasionally intercalary, predominately paragynous, sometimes amphigynous, barrel- or club-shaped to irregular	aplerotic, spherical	Naher et al. 2011
mostly spherical	predominately paragynous, sometimes amphigynous	aplerotic, spherical	Tomić and Ivić 2015
spherical, predominately rounded base	predominately paragynous, sometimes amphigynous, one per oogonium	aplerotic, spherical	Götz et al 2017 Werres, unpublished data
N/A	amphigynous, paragynous	aplerotic	Lin et al. 2017

N/A = no information available

Tab. 3: Sequence analysis of *Phytophthora chrysanthemi* isolates [1]

Isolates from	Isolates studied	DNA database accession no.							References
		ITS rDNA	28S rDNA	mt Cytochrome c oxidase	Elongation factor 1α [2]	β-tubulin	Heat shock protein 90	60S Rib protein L10	
Japan	GF753	AB511826	AB511313	N/A	AB511927	AB511996	AB511988	AB511934	Naher et al. 2011
	Chr5	AB511827	AB511314	N/A	AB511929	AB511998	AB511990	AB511936	Naher et al. 2011
	Chr3	AB437136	AB465508	AB465510 [4]	AB511928	AB511997	AB511989	AB511935	Naher et al. 2011
	JKI-026-16-8-00-0-0	MG601075	MG601058	MG601081 [3]	MG601087	MG601067	N/I	N/I	Götz, unpublished data
	GF749	AB437135	AB465349	AB465509 [4]	AB511925	AB511995	AB511987	AB511926	Naher et al. 2011
	CBS 123163	KT183038	N/A	N/A	N/A	N/A	N/A	N/A	NCBI
	JKI-027-16-8-00-0-0	MG601076	MG601059	MG601082 [3]	MG601088	MG601068	N/I	N/I	Götz, unpublished data
	MAFF 712282	AB688343	AB688485	AB688212 [3]	N/A	N/A	N/A	N/A	Rahman et al. 2014
Croatia	PH_47	KJ508824	N/A	N/A	N/A	N/A	N/A	N/A	Tomić and Ivić 2015
USA	JKI-009-17-8-00-0-0	MG601077	MG601060	MG601083 [3]	MG601089	MG601069	N/I	N/I	Götz, unpublished data
	JKI-010-17-8-00-0-0	MG601078	MG601061	MG601084 [3]	MG601090	MG601070	N/I	N/I	Götz, unpublished data
	FPH2016-102	KY412775	N/I	N/I	N/I	N/I	N/I	N/I	Lin et al. 2017
	JKI-011-17-8-00-0-0	MG601079	MG601062	MG601085 [3]	MG601091	MG601071	N/I	N/I	Götz, unpublished data
Germany	JKI-050-15-8-01-2-0	KY363520	KY363521	KY363522 [3]	KY363524	KY363523	MG601073	MG601065	Götz et al. 2017
	JKI-050-16-8-01-2-0	MG601074	MG601063	MG601080 [3]	MG601086	MG601066	MG601072	MG601064	Götz, unpublished data

N/I = not studied, N/A = no information available
[1] Sequence data of further but unpublished strains in NCBI available
[2] The sequences highlighted in pale green have identities >99.5% to each other but only <96.7% to the sequences highlighted in pale red
[3] mtCOX I, partial
[4] mtCOX II, partial; accession numbers are not cited in Naher et al. 2011

Diagnosis

Direct Isolation

- Easy with samples from the necrotic stem base, difficult with root samples
- The following agar media are favorable for *P. chrysanthemi*:

  Carrot Piece agar (CPA): 50 g carrot pieces, 15 g agar, 1000 ml ultrapure water

  Chrysanthemum agar (ChryA): 60 g macerated fresh leaves from *Chrysanthemum indcum,* 15 g agar, 1000 ml ultrapure or *a. dest*. water,
  this agar is favorable especially for oospore induction

  Lima Bean agar (LBA): 75 ml Lima Bean filtrate, 20 g agar; fill up to 1000 ml with D.I. water; autoclave. *To prepare filtrate*: 100mL water + 50 grams frozen lima beans and autoclave. Following sterilization, mash up lima beans in a sterile mortar with pestle. Place 2 layers of cheese cloth over the top of a flask and pour mixture through the cloth.

  V8 +$CaCO_3$ agar (1): 200 ml V8 juice, 2.8 g $CaCO_3$, 20 g agar; fill up to 1000 mL with *a. dest*.

  V8+$CaCO_3$ agar (2): 100 ml V8 juice, 1 g $CaCO_3$ [CAS no 471-34-1], 15 g agar; fill up to 1000 mL with ultrapure water or *a. dest*.
- Incubation at 25°C is favorable
- *P. chrysanthemi* is a slow growing *Phytophthora* species and can degenerate when subcultered
- A variety of different agar media can be used for morphological studies, and the preference can depend on the isolate

Tab. 4: Influence of type of the agar medium on *P. chrysanthemi* growth rate and propagule development

Isolates studied	Vegetative growth rate at optimum temperature (mm/24h) on		Sporangia development on		Chlamydospore development on		Oospore development on		
	V8 + $CaCO_3$ agar	CPA	V8 + $CaCO_3$ agar	CPA	V8 + $CaCO_3$ agar	CPA	V8 + $CaCO_3$ agar	CPA	ChryA
JKI-009-17-8-00-0-0	3.9*	3.2	Ø	Ø	Ø	X	Ø	X	X
JKI-010-17-8-00-0-0	2.8*	3.4	Ø	Ø	Ø	X	Ø	X	X
JKI-011-17-8-00-0-0	2.5	1.5	Ø	Ø	X	X	Ø	X	X

*degeneration of the hyphae after 2 weeks, CPA= Carrot Piece Agar, ChryA = *Chrysanthemum* Agar,
X = present, Ø = absent

Microscopy of the roots

P. chrysanthemi develops oospores in infected roots (Fig. 3)

Bait tests for soil, substrate and water

- Very easy with rooted cuttings of *Chrysanthemum indicum* (Götz and Werres unpublished data), you only have to plant the rooted cuttings into the substrate/soil
- *Chrysanthemum* leaves are also very good baits (Tomić and Ivić, unpublished data)
- Rhododendron leaves seem not to be a good bait for *P. chrysanthemi*

Serological methods (z.B. Schnelltests)

ImmunoStrip test (Agdia, Inc., https://orders.agdia.com/agdia-immunostrip-for-phyt-isk-92601) (Lin et al. 2017)

PCR

Specific primers for *P. chrysanthemi* are not yet available

Recommendation

- Examination of samples with at least two different diagnostic techniques, e.g. for plant samples. PCR with genus specific primers and can give a first indication on a *Phytophthora* infection. Similar with commercially available ELISA kits for *Phytophthora* detection that are genus specific.
- Determination of the *Phytophthora* species in pure agar cultures by morphology and by sequence analysis.
- For differences between the closely related *P. parsiana* see Naher et al. (2011) and *P. cuyabensis* see Rahman et al. (2014).

Help with diagnosis

In Germany: Sabine WERRES, sabine.werres@julius-kuehn.de

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In USA: Francesca PEDUTO HAND, hand.81@osu.edu

What to do in case plants are suspected to be infected?

Please contact your national authorities.

Examples for **European contact addresses**: addresses.pdf

In **Germany** please contact first your plant protection services; address list see https://www.julius-kuehn.de/linksammlung/

In the **USA**, each state has an in-state based plant disease clinic. To find a lab based on state, visit: https://www.npdn.org/. In Ohio, you can contact the C.W. Ellett Plant and Pest Diagnostic Clinic – The Ohio State University at https://ppdc.osu.edu/

Risk analysis

For Germany:
http://pflanzengesundheit.jki.bund.de/dokumente/upload/348cb_phytophthora-chrysanthemi_express-pra_en.pdf

For the USA:
http://www.canr.org/newsletter/PhytophthorachrysanthemiNPAGReport20160401R.pdf

Literature

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Internet

- JKI Data Sheet '*Phytophthora* on *Chrysanthemum*' (chrysants, mums)
- EPPO Global Database: https://gd.eppo.int/search?k=Phytophthora+chrysanthemi
- http://phytophthora-id.org/
- http://www.canr.org/newsletter/PhytophthorachrysanthemiNPAGReport20160401R.pdf

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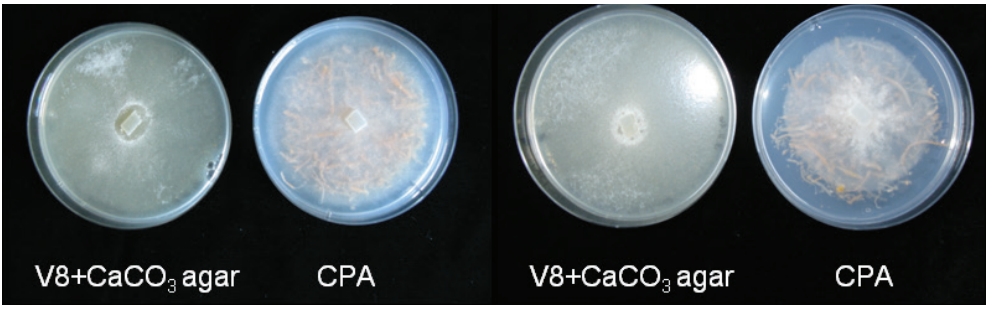
Phytophthora chrysanthemi



Japanese isolate JKI-027-16-8-00-0-0 German isolate JKI-050-15-8-01-2-0

Fig.1. Colony pattern of *P. chrysanthemi* (11 days after incubation in the dark at 28°C)

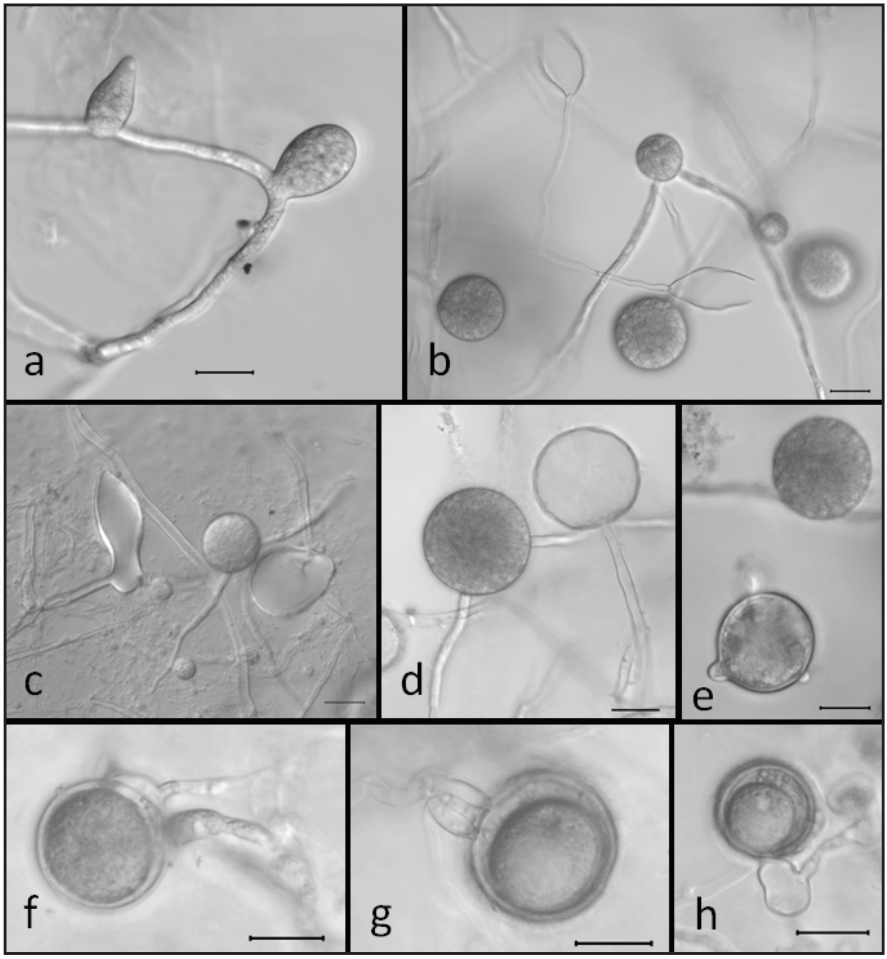


Fig. 2. Morphological characteristics of *P. chrysanthemi* JKI-050-15-8-01-2-0

a – Hyphal swellings
b – Chlamydospores and empty sporangia
c – Intercalar chlamydospore, empty sporangium and
d – Intercalar and terminale chlamydospore
e – Germinating chlamydospore
f – Oogonium with paragynous antheridium
g – Oogonium with amphigynous antheridium
h – Aplerotic oospores

a-e on CPA or V8+$CaCO_3$ agar, f-h on *Chrysanthemum* agar

Scale bar = 20 µm

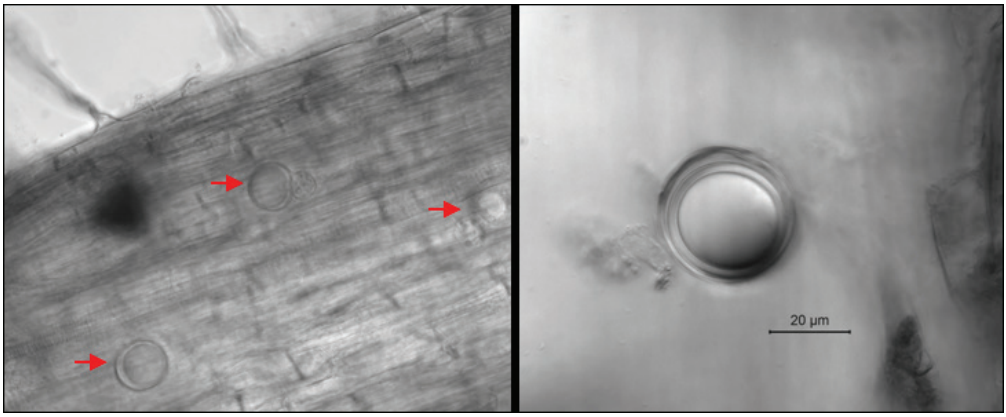


Fig. 3. Oospores of *P. chrysanthemi* in naturally infected roots of *Chrysanthemum indicum*

Photos: S. WERRES, M. GÖTZ