Biological efficacy of a biorationals based on sea weed and humic acid to control potato late blight

Pot experiment KAS 973 October 2018

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This study was carried out by the Wageningen Research Foundation (WR) business unit Field Crops and was commissioned and financed by PPS AF 16002 MIP.

WR is part of Wageningen University & Research, the collaboration of Wageningen University and Wageningen Research Foundation.

Wageningen, January 2019

WPR-OT 936

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A. Evenhuis & C.G. Topper, 2019. *Biological efficacy of a biorationals based on sea weed and humic acid to control potato late blight.* Wageningen Research, Report WPR-OT 936

This report can be downloaded for free at https://doi.org/10.18174/570732.

Contents

1 Introduction

Late blight, caused by *Phytophthora infestans*, is the most important disease in potato production because of its capacity to destroy foliage very rapidly and its ability to infect tubers. To avoid infection of the foliage, fungicides are used frequently. In the Netherlands, in some years up to 15 sprays are necessary to prevent the crop from infection by the pathogen. In the Netherlands tomato is a crop grown in greenhouses. In the Mediterranean, tomatoes are grown in the open field and can suffer from late blight as well.

Efficient use of crop protection agents is desirable. Improvement of the efficacy of active ingredients is a possibility to reduce environmental burden and at the same time achieve better results to control late blight in the field. Environmental burden could also be lowered by introducing biorationals in the control strategy.

The efficacy of fungicides and biorationals to control late blight depends on their characteristics, dose rate and the time between the spray application and the infection of *P. infestans*.

In this experiment the efficacy of sea weed and humic acid at two dose rates against *P. infestans* was tested in a pot experiment in comparison with mancozeb. The experiment was carried out on tomatoes because potatoes are not easily grown late in autumn. Nevertheless, the experiment aimed to resemble agricultural practise as close as possible within an experimental set-up. However since the test was carried out in the second half of the year tomato was chosen as host species instead of potato. The experiment was carried out by request of ...

2 Methods and materials

2.1 Experimental set up

The cultivated tomato plants (cv. Albis) were grown in pots. The experiment was carried out in accordance with GEP (NVWA-recognition; Appendix 1) The experiment was artificially inoculated.

Conducted Under GLP: No Official Trial ID: -
Conducted Under GEP: Yes Other Trial ID: KAS973

No.	Guideline	Description
1.	PP 1/135(4)	phytotoxicity assessment
2.	PP 1/152(4)	Design and analysis of efficacy evaluation trials
3.	PP 1/181(4)	Conduct and reporting of efficacy evaluation trials including GEP
4.	PP 1/2(4)	Phytophthora infestans on potato

2.2 Treatments

In Table 1 the fungicides used and dose rates are presented. The fungicide sprayings were carried out when the tomato plants reached a height of 25-30 cm. The tomato plants were sprayed in a spraying cabin developed by Wageningen University & Research business unit Field Crops. The fungicides were sprayed using a spray boom with three spray nozzles Airmix 110-03 at 2.5 bar, placed 50 cm apart, which was moving with a speed of 5 km/h approximately 40 cm over the top of the tomato plants. Spray volume was 250 l/ha. Spray application were carried out on 18 and 24 October 2018.

Table 1 Treatments and fungicides applied 1 or 7 days before inoculation

Code	Fungicide	Active ingredient	Dose rate l or kg per ha	Spray application
A	UTC	-	-	-
B	Dithane DG NT	mancozeb 750 g/kg	2.0	- 1 day
N			1.5	- 1 day
O			3.0	- 1 day
S			3.0	-7 days
P			1.0	- 1 day
R			2.0	- 1 day
T			2.0	-7 days

2.3 Inoculation *P. infestans*

The inoculation procedure was carried out according to a standard protocol that is used for all late blight experiments. A *P. infestans* isolate belonging to the EU-13-A2 (Blue13) clonal lineage was used. This isolate was chosen because it belongs to the most important genetic group of *P. infestans* in Europe. The isolate was stored in liquid nitrogen at Wageningen University & Research business unit BioInteractions & Plant Health. From the isolate a plate culture was made. The inoculum suspension was made by rinsing a one week old culture of *P. infestans* with water. The inoculum density was set at approximately 3.000 spores/ml. Inoculation was carried out by spraying tomato plants over head with approximately 10 ml of inoculum per plant. Inoculation was carried out on air dry plants. The experiment was inoculated on 25 October 2018.

2.4 Disease observations

Disease observations were carried out three times. The percentage necrotic foliage of 4 leaves per plant was estimated visually. Average disease severity was calculated per assessment date and over the three assessment dates.

2.5 Statistics

Analysis of variance on the parameters was made using GENSTAT 19th Edition. The experiment was carried out with six replications. Each replication consisted of a potted tomato plant. Transformation of data was carried out when necessary.

3 Results

No phytotoxicity on the tomato plants after spray application of the products was observed. The results are presented in Table 2, Figure 1 and Figure 2. In Appendix 2 the detailed data are presented.

Late blight severity was significantly lower than the untreated control for all treatments tested regardless of the dose rate or the spray interval. Based on the average of three disease assessments the efficacy to control potato late blight of the reference treatment was significantly better than treatments, N, O, S, P, R and T. The efficacy of treatment R to control potato late blight was significantly better than treatments N, O, S, P and T. The efficacy to control potato late blight of treatments P and T were significantly better than treatments N, O and S. The efficacy of Humison treatments acid (N, O & S) were comparable and no significant dose rate and spray interval effect was found. A dose rate effect and a spray interval effect was found when applying Seamel, compare R vs P and T.

Table 2 Late blight severity (%) at 4, 6 and 8 days after inoculation (dpi) and on average (Phy%avg), and percentage control of *P. infestans* based on average.[2]

label[1]	Phytin 4 dpi	Phytin 6 dpi	Phytin 8 dpi	Phytin average
UTC	24.3	48.8	71.7	48.2
reference	0.0	0.0	0.04	0.01
N	10.2	18.7	29.8	19.6
O	14.8	26.6	39.3	26.9
S	14.8	25.8	41.3	27.3
P	6.4	8.3	16.1	10.3
R	5.0	5.0	9.8	6.6
T	7.2	10.5	15.9	11.2
Lsd	3.0	8.1	10.9	6.9
F pr.	<0.001	<0.001	<0.001	<0.001

label[2]	Phytin 4 dpi	Phytin 6 dpi	Phytin 8 dpi	Phytin average
UTC	24.2 f[3]	48.5 e	71.4 e	48.1 e
reference	0.0 a	0.0 a	0.04 a	0.01 a
N	9.8 d	18.4 d	29.3 d	19.3 d
O	14.6 e	25.3 d	36.3 d	25.8 d
S	13.8 e	23.5 d	39.3 d	25.7 d
P	6.1 bc	8.1 c	15.2 c	9.9 c
R	4.9 b	4.9 b	9.8 b	6.6 b
T	7.0 c	9.4 c	14.6 c	10.5 c
Lsd	-	-	-	-
F pr.	<0.001	<0.001	<0.001	<0.001

[1]) The upper table gives the arithmetical means, when followed by a character the values are normally distributed allowing ANOVA without transformation.

[2]) The lower table gives the log10 (x+1) back transformed values to meet the requirements for a normal distribution of the data.

[3]) Values in columns followed by the same character are not significantly different (P=0.05).

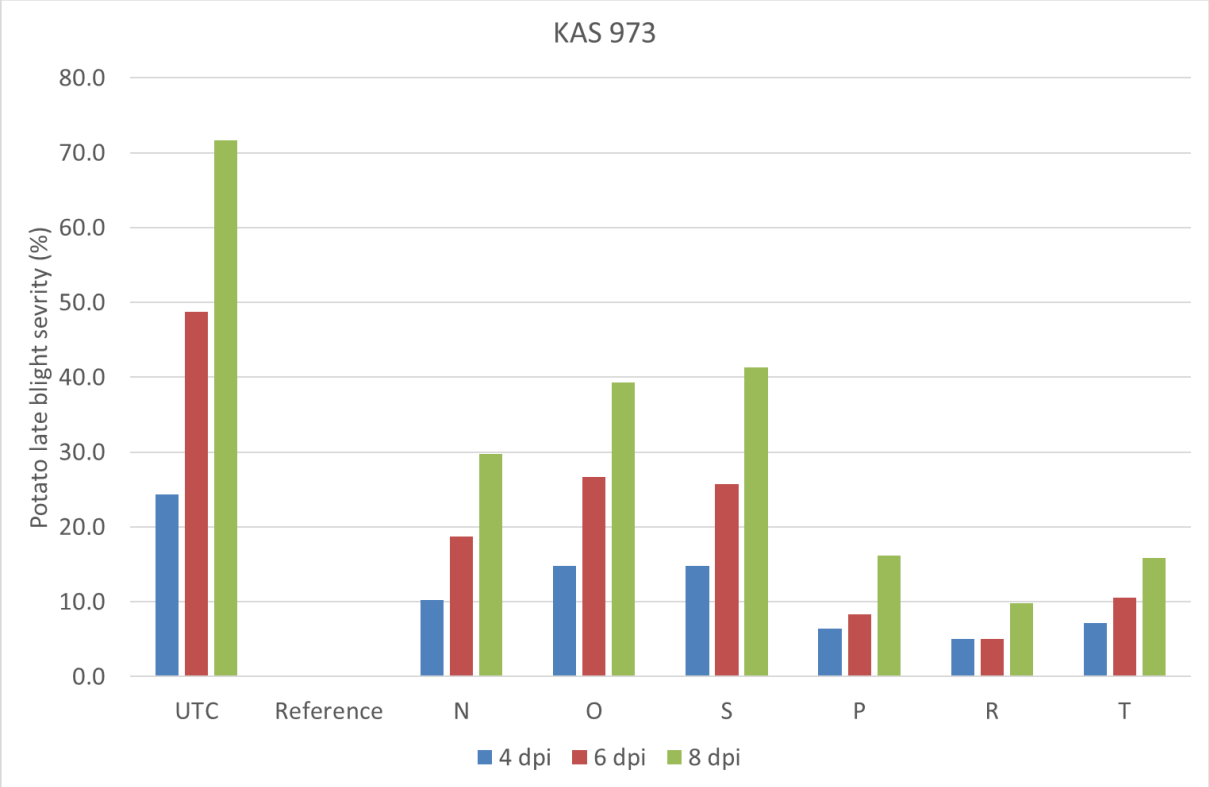

Figure 1 Late blight severity 4, 6 and 8 days past inoculation on tomato leaves.

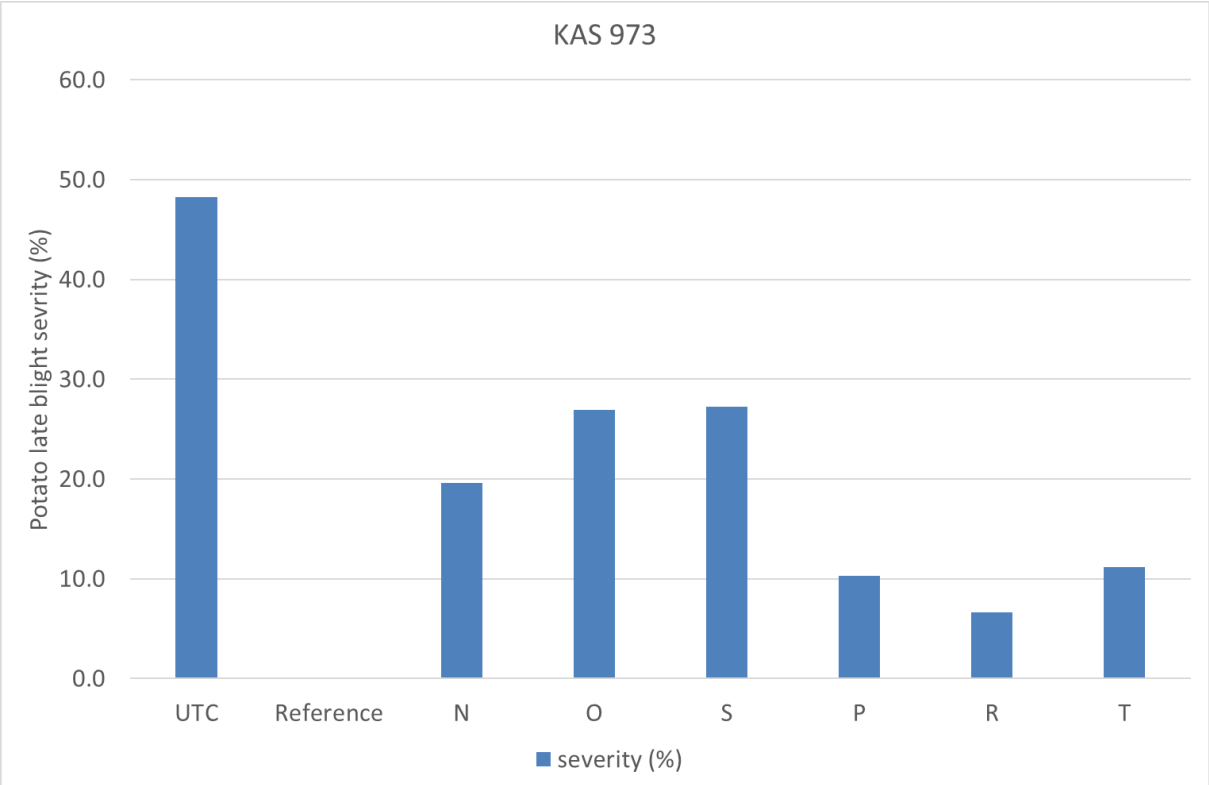

Figure 2 Average late blight severity (average of 3 assessments) after inoculation on four leaves per plant.

Figure 3 Late blight severity on tomato from left to right treatments A, B, N, O, P, R, S and T. Picture taken on 2 November 2018. Eight days after inoculation.

4 Discussion and conclusion

4.1 Discussion

The untreated control (UTC) was readily infected by *P. infestans* indicating that the artificial inoculation was successful.
The experiment was inoculated with an isolate belonging to the clonal lineage EU-13-A2 (Blue13). Blue13 is generally accepted as an aggressive *P. infestans* type on potato, although new and probably more aggressive isolates are found in the *P. infestans* population. Since we were testing biorationals and not fungicides we chose to lower the disease pressure by inoculating 3.000 spores / ml instead of the normal 10.000 spores in such an experiment. The aim was to have an indication of whether the product showed some efficacy to control potato late blight. The consequence of lowering the dose rate was that the reference treatment was not infected by *P. infestans* except for 1 lesion.
Thus, on the one hand the disease pressure was relatively low. On the other hand the tomato cultivar chosen was probably very susceptible since the untreated control was highly infected. Since the performance of both Humison and Seamel to control potato late blight was less than Dithane DG NT it is not recommend to use these product to control potato late blight in very susceptible varieties, especially if fungicide application is allowed.
Fortunately also less susceptible cultivars to *P. infestans* are grown in The Netherlands. Not only on organic production but also in conventional potato cultivation. It has been shown that cultivar resistance and fungicide dose rates applied have an additive effect. This means that when the cultivar is less susceptible the dose rate of a fungicide can be lowered. It would be interesting to investigate whether the products would be able to control potato late blight in less susceptible cultivars as a replacement for fungicides. Possibly not at high infection risk but maybe at moderate infection risks. For instance in the PPS GROEN incorporation of biorationals in control is strategies is investigated with the aim to apply biorationals regularly to control diseases and fungicides only at high infection risk.
In case of organic cultivation possibly the products could be applied to delay the potato late blight epidemic. It would be interesting to investigate whether such a delay is feasible and under what conditions.
It is not known if the effect of Humison and Seamel is directly on *P. infestans*, or for instance is a result of plant strengthening or induced resistance. The fact that both products showed efficacy seven days after application is promising for future incorporation in agriculture.

4.2 Conclusions

- No phytotoxicity was observed, the products sprayed were crop safe.
- Late blight severity was significantly lower than the untreated control for all treatments tested regardless of the dose rate or the spray interval.
- Based on the average of three disease assessments the efficacy to control potato late blight of the reference treatment was significantly better than treatments, N, O, S, P, R and T.
- The efficacy of treatment R to control potato late blight was significantly better than treatments N, O, S, P and T.
- The efficacy to control potato late blight of treatments P and T were significantly better than treatments N, O and S.
- The efficacy of Humison treatments acid (N, O & S) were comparable and no significant dose rate and spray interval effect was found.
- A dose rate effect and a spray interval effect was found when applying Seamel, compare R vs P and T.

Annex 1 NVWA Certificate

Netherlands Food and Consumer
Product Safety Authority
Ministry of Economic Affairs

Certificate

of Official Recognition of Efficacy Testing Organisations in the Netherlands
This certifies that, in conformity with the request of March 9, 2017

STICHTING WAGENINGEN RESEARCH
BUSINESS UNIT PRAKTIJKONDERZOEK AGV

Residing: Edelhertweg 1 Lelystad, the Netherlands

has officially been recognised as an organisation for efficacy testing in the Netherlands.

As has been laid down in the 'Regeling gewasbeschermingsmiddelen en biociden' (Regulation Crop Protection Products and Biocides) of September 26, 2007 (Staatscourant 2007, 386).

This recognition commences on: March 1, 2017
and expires on: February 12, 2022

The above organisation is competent to carry out efficacy trials/tests in the categories mentioned in the annex of this certificate.

Utrecht, March 14, 2017

For the Minister of Economic Affairs,

Ir. W.J.H. van der Sande
Deputy Director
Netherlands Plant Protection Organization

CERTIFICATE NUMBER: NL_GEP_13160822 Page 1 of 2

Annex 2 Disease observations

Datum:					29-10	29-10	29-10	29-10	
Opmerkingen:									
Doel:					Phytophthora infestans	Phytophthora infestans	Phytophthora infestans	Phytophthora infestans	
Beoordeling niveau:					Samengeseld b	Samengeseld b	Samengeseld b	Samengeseld blad 4	
Beoordeling:					Blad	Blad	Blad	Blad	
Eenheid:					%	%	%	%	
Manier:					Schatten	Schatten	Schatten	Schatten	
Potnr	Code	Dose	Spray timin	Blok					PHYTIN AVG
Potnr!	Object!	Dosering	uit tijdstipp	Blok!	Blad12910	Blad22910	Blad32910	Blad42910	Phy4dpi
101	P	1	-1	1	8	7	8	10	8.25
102	O	3	-1	1	7	20	17	17	15.25
103	S	3	-7	1	15	20	20	17	18
104	R	2	-1	1	6	6	9	5	6.5
105	T	2	-7	1	7	12	7	7	8.25
106	N	1.5	-1	1	12	15	15	9	12.75
107	A	0	-1	1	20	30	30	30	27.5
108	B	2	-1	1	0	0	0	0	0
109	S	3	-7	2	25	30	25	17	24.25
110	R	2	-1	2	4	4	4	6	4.5
111	A	0	-1	2	25	35	25	20	26.25
112	B	2	-1	2	0	0	0	0	0
113	N	1.5	-1	2	7	12	12	10	10.25
114	T	2	-7	2	8	8	6	4	6.5
115	P	1	-1	2	8	6	9	9	8
116	O	3	-1	2	17	20	17	20	18.5
117	A	0	-1	3	17	25	25	25	23
118	P	1	-1	3	9	10	8	8	8.75
119	S	3	-7	3	20	20	20	6	16.5
120	O	3	-1	3	9	17	15	15	14
121	T	2	-7	3	20	8	8	5	10.25
122	R	2	-1	3	5	6	6	5	5.5
123	N	1.5	-1	3	8	7	12	10	9.25
124	B	2	-1	3	0	0	0	0	0
125	N	1.5	-1	4	12	18	15	15	15
126	B	2	-1	4	0	0	0	0	0
127	P	1	-1	4	3	4	5	4	4
128	O	3	-1	4	8	15	17	8	12
129	R	2	-1	4	3	3	3	3	3
130	S	3	-7	4	18	12	10	9	12.25
131	T	2	-7	4	6	6	6	3	5.25
132	A	0	-1	4	25	20	25	20	22.5
133	A	0	-1	5	20	25	25	25	23.75
134	T	2	-7	5	10	8	7	7	8
135	S	3	-7	5	9	9	8	9	8.75
136	O	3	-1	5	9	15	15	10	12.25
137	B	2	-1	5	0	0	0	0	0
138	N	1.5	-1	5	8	7	7	8	7.5
139	P	1	-1	5	4	7	4	5	5
140	R	2	-1	5	8	4	4	7	5.75
141	P	1	-1	6	5	5	4	3	4.25
142	B	2	-1	6	0	0	0	0	0
143	S	3	-7	6	8	12	9	6	8.75
144	R	2	-1	6	4	6	5	5	5
145	N	1.5	-1	6	6	8	5	7	6.5
146	A	0	-1	6	1	40	25	25	22.75
147	O	3	-1	6	15	20	20	12	16.75
148	T	2	-7	6	5	4	5	5	4.75

Datum:		31-10	31-10	31-10	31-10	31-10
Opmerkingen:						
Doel:		Phytophthora infestans	Phytophthora infestans	Phytophthora infestans	Phytophthora infestans	
Beoordeling niveau:		Samengeseld b	Samengeseld b	Samengeseld b	Samengeseld	blad 4
Beoordeling:		Blad	Blad	Blad	Blad	
Eenheid:		%	%	%	%	
Manier:		Schatten	Schatten	Schatten	Schatten	
Potnr	Code					PHYTIN AVG
Potnr!	Object!	Blad13110	Blad23110	Blad33110	Blad43110	Phy6dpi
101	P	8	7	12	15	10.5
102	O	6	18	15	20	14.75
103	S	15	25	25	25	22.5
104	R	4	4	6	6	5
105	T	7	8	8	8	7.75
106	N	18	20	18	20	19
107	A	30	55	55	60	50
108	B	0	0	0	0	0
109	S	50	60	55	45	52.5
110	R	4	5	6	8	5.75
111	A	35	55	60	55	51.25
112	B	0	0	0	0	0
113	N	12	18	20	30	20
114	T	4	6	5	4	4.75
115	P	8	7	9	9	8.25
116	O	20	30	25	25	25
117	A	25	35	45	50	38.75
118	P	7	8	7	8	7.5
119	S	30	35	30	15	27.5
120	O	10	25	20	25	20
121	T	25	8	10	9	13
122	R	3	4	5	5	4.25
123	N	12	18	20	12	15.5
124	B	0	0	0	0	0
125	N	12	25	30	35	25.5
126	B	0	0	0	0	0
127	P	4	5	5	5	4.75
128	O	10	30	35	25	25
129	R	4	3	3	3	3.25
130	S	20	15	30	18	20.75
131	T	10	7	6	4	6.75
132	A	40	60	45	45	47.5
133	A	50	55	60	55	55
134	T	25	18	25	18	21.5
135	S	10	9	20	18	14.25
136	O	20	45	55	35	38.75
137	B	0	0	0	0	0
138	N	15	12	15	15	14.25
139	P	4	15	4	15	9.5
140	R	8	7	5	10	7.5
141	P	9	7	15	7	9.5
142	B	0	0	0	0	0
143	S	18	25	15	10	17
144	R	4	5	4	5	4.5
145	N	20	20	12	20	18
146	A	45	50	50	55	50
147	O	25	55	35	30	36.25
148	T	9	8	12	8	9.25

Datum:		2-11	2-11	2-11	2-11	2-11	
Opmerkingen:							
Doel:		Phytophthora infestans	Phytophthora infestans	Phytophthora infestans	Phytophthora infestans		
Beoordeling niveau:		Samengeseld b	Samengeseld b	Samengeseld b	Samengeseld blad 4		
Beoordeling:		Blad	Blad	Blad	Blad		
Eenheid:		%	%	%	%		
Manier:		Schatten	Schatten	Schatten	Schatten		
Potnr	Code					PHYTIN AVG	
Potnr!	Object!	Blad10211	Blad20211	Blad30211	Blad40211	Phy8dpi	Phy%avg
101	P	12	10	25	30	19.25	12.7
102	O	7	25	20	15	16.75	15.6
103	S	30	40	35	30	33.75	24.8
104	R	4	6	15	20	11.25	7.6
105	T	8	10	8	9	8.75	8.3
106	N	20	35	30	40	31.25	21.0
107	A	60	80	75	85	75	50.8
108	B	0	0	1	0	0.25	0.1
109	S	65	80	70	65	70	48.9
110	R	6	6	7	15	8.5	6.3
111	A	45	85	85	70	71.25	49.6
112	B	0	0	0	0	0	0.0
113	N	20	30	30	35	28.75	19.7
114	T	6	15	12	8	10.25	7.2
115	P	20	20	25	30	23.75	13.3
116	O	30	40	35	45	37.5	27.0
117	A	25	60	70	80	58.75	40.2
118	P	9	18	12	18	14.25	10.2
119	S	45	40	50	25	40	28.0
120	O	15	30	25	35	26.25	20.1
121	T	35	20	30	20	26.25	16.5
122	R	4	7	12	12	8.75	6.2
123	N	20	30	30	25	26.25	17.0
124	B	0	0	0	0	0	0.0
125	N	15	35	50	50	37.5	26.0
126	B	0	0	0	0	0	0.0
127	P	4	8	9	8	7.25	5.3
128	O	15	60	75	35	46.25	27.8
129	R	10	8	8	9	8.75	5.0
130	S	35	40	45	65	46.25	26.4
131	T	10	12	12	7	10.25	7.4
132	A	60	80	80	70	72.5	47.5
133	A	65	80	85	85	78.75	52.5
134	T	25	20	20	30	23.75	17.8
135	S	25	25	35	35	30	17.7
136	O	25	70	60	40	48.75	33.3
137	B	0	0	0	0	0	0.0
138	N	20	20	20	25	21.25	14.3
139	P	15	30	12	18	18.75	11.1
140	R	15	9	7	18	12.25	8.5
141	P	15	12	15	12	13.5	9.1
142	B	0	0	0	0	0	0.0
143	S	30	45	25	12	28	17.9
144	R	7	12	10	9	9.5	6.3
145	N	30	35	30	40	33.75	19.4
146	A	70	80	75	70	73.75	48.8
147	O	40	75	70	55	60	37.7
148	T	12	12	20	20	16	10.0

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