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Alabama04-1         ATGGCCAAGATTATTATTGCCGTTATACCTGCTGATCAGGATGCTAGACTTAAGGCAAAG 60
India73-1           ATGGCCAAGATTATTATTGCCGTTATACCTGCTGATCAGGATGCTAGACTAAAGGCAAAG 60
SouthAfrica01-1     ATGGCCAAGATTATTATTGCCGTTATACCTGCTGATCAGGATGCTAGACTTAAGGCAAAG 60
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Thailand02-1        ATGGCCAAGATTATTATTGCCGTTATACCTGCTGATCAGGATGCTAGACTTAAGGCAAAG 60
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India73-1           GCCCAGACCTGGCATTGAATTTTTGTCTATAGGCTTTGATAATTGATTTTTTTAAATGCT 180
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India73-1           TTTTTTTACTATTGCAGTTGTTGTAGTCAGCGACCCTAGCAAAGTTGTTGAGACCATTAA 240
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Paraguay01-3        TTTTTTTACTATTGCAGTTGTTGTAGTCAGCGACCCTAGCAAAGTTGTTGAGACCATTAA 240
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Taiwan72-1          GGCCCAGCCAGAGCCTCCAGTTGTTGTTGTAAGTTTTGAAAATTATGGCTACCTTTTCCT 300
Alabama04-1         GGCCCAGCCAGAGCCTCCAGTTGTTGTTGTAAGTTTTGAAAATTATGGCTACCTTTTCCT 300
India73-1           GGCCCAGCCAGAGCCTCCAGTTGTTGTTGTAAGTTTTGAAAATTATGGCTACCTTTTCCT 300
SouthAfrica01-1     GGCCCAGCCAGAGCCTCCAGTTGTTGTTGTAAGTTTTGAAAATTATGGCTACCTTTTCCT 300
Paraguay01-3        GGCCCAGCCAGAGCCTCCAGTTGTTGTTGTAAGTTTTGAAAATTATGGCTACCTTTTCCT 300
Thailand02-1        GGCCCAGCCAGAGCCTCCAGTTGTTGTTGTAAGTTTTGAAAATTATGGCTACCTTTTCCT 300
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Taiwan72-1          CGCTACTTCTTCAATTATTTATAGTTTTCTTATCTCTTTGAAATTTGGTTTTACTTTTCT 360
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SouthAfrica01-1     CGCTATTTCTTCAATTATTTATAGTTTTCTTATCTCTTTGAAATTTGGTTTTACTTTTCT 360
Paraguay01-3        CGCTATTTCTTCAATTATTTATAGTTTTCTTATCTCTTTGAAATTTGGTTTTACTTTTCT 360
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SouthAfrica01-1     ACTCTTAAAGTCCGTCACCAACTTTTTCTCTGAGAGCGTCCAACAAGAAGTCAAGAAGGC 420
Paraguay01-3        ACTCTTAAAGTCCGTCACCAACTTTTTCTCTGAGAGCGTCCAACAAGAAGTCAAGAAGGC 420
Thailand02-1        ACTCTTAAAGTCCGTCACCAACTTTTTCTCTGAGAGCGTCCAACAAGAAGTCAAGAAGGC 420
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India73-1           CGTCGAGGCAGGTGCCCCAGGTGTACCTGTTCTCCTTGTACCTCAAGGATTAGACGACGA 480
SouthAfrica01-1     CGTCGAGGCAGGTGCCCCAGGTGTACCTGTTCTCCTTGTACCTCAAGGATTAGACGACGA 480
Paraguay01-3        CGTCGAGGCAGGTGCCCCAGGTGTACCTGTTCTCCTTGTACCTCAAGGATTAGACGACGA 480
Thailand02-1        CGTCGAGGCAGGTGCCCCAGGTGTACCTGTTCTCCTTGTACCTCAAGGATTAGACGACGA 480
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Taiwan72-1          ACAGGTCTACGAATTCTTAAAGGCAGAGAAAGCCAAACATTCT 523
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SouthAfrica01-1     ACAGGTCTACGAATTCTTAAAGGCAGAGAAAGCCAAACATTCT 523
Paraguay01-3        ACAGGTCTACGAATTCTTAAAGGCAGAGAAAGCCAAACATTCT 523
Thailand02-1        ACAGGTCTACGAATTCTTAAAGGCAGAGAAAGCCAAACATTCT 523
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Fig. S1 Sequence alignment of PHEP 369 genomic DNA isolates. Sequences were aligned using CLUSTAL 2.0.12.

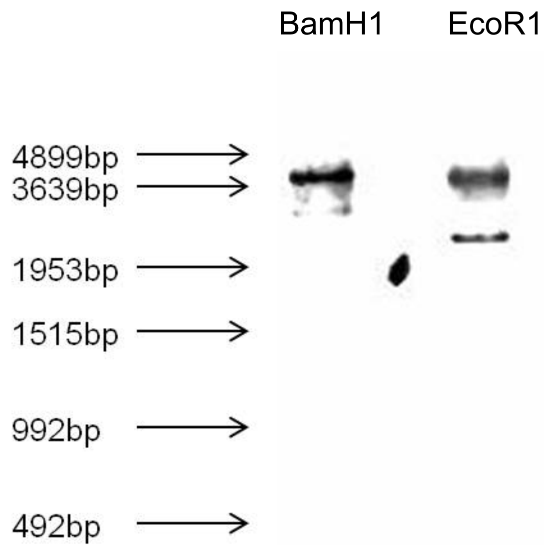


Fig. S2. Southern Blot Analysis of PHEP 369 - Genomic DNA was extracted from *P. pachyrhizi* isolate TW72-1 as previously described. Ten µg of DNA was digested with EcoR1 and BamH1, run on an agarose gel and transferred onto Nylon membrane. A full length PHEP 369 genomic DNA probe was generated using a DIG PCR labeling kit (Roche Applied Science, Indianapolis, IN) with the following primer set (5'-ATG GGA AAA GTT ATC ATC AAT GTG-3' and 5'-CTT TCC AGC CTT TGC TTT TTC ATC-3'). Hybridization was carried out using DIG Easy Hyb solution according to manufacture's protocol (Roche Applied Science) at 40° C for 16 hr. Blots were washed in 2X SSC and 0.01% SDS at 55° C and evaluated using the DIG detection system with CDP-STAR (Roche Applied Science) chemiluminescence**.**

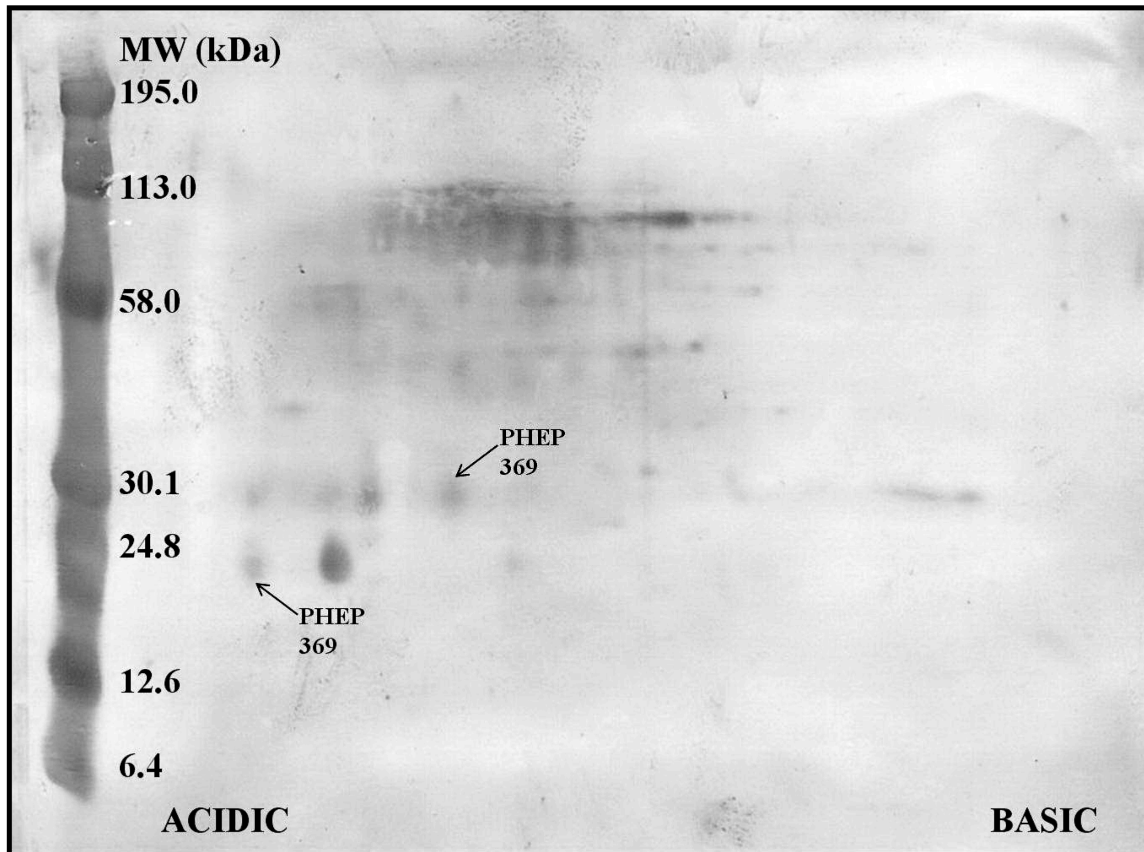


Fig. S3. Lectin affinoblot of *P. pachyrhizi* proteins. Identification of PHEP 369 proteins were deduced by comparison of molecular weight and pI to spots identified on colloidal blue-stained 2-D gel of *P. pachyrhizi* urediniospore proteins (for 2-D gel, see http://world-2dpage.expasy.org/repository/database=0018)

Methods and Materials

Protein Extraction and 2-D PAGE –was performed as described by Luster et al. (2010).

Lectin affinoblotting and detection of lectin conjugates. Proteins were transferred from 2-D gels as described for immunoblotting. After transfer, blots were blocked in 3% (w/v) casein in PBS plus 1 mM $MnCl_2$ and 1 mM $CaCl_2$ (cations) for 1 hour, and subsequently probed with biotin-labelled concanavalinA (Sigma Chemical Co., St. Louis, MO) for 1 hour at 10 µg/mL in 0.3% casein + cations (Faye and Chrispeels, (1985). Blots were washed for 15 minutes three times in 100 mLPBS-0.02% Tween-20. Blots were then probed with streptavidin conjugated to alkaline phosphatase (Roche Applied Science, Indianapolis, IN) in PBS + cations at 1:2000 dilution for 1 hour. Alkaline phosphatase was detected with kit reagents according to the manufacturer's protocols (Sigma).

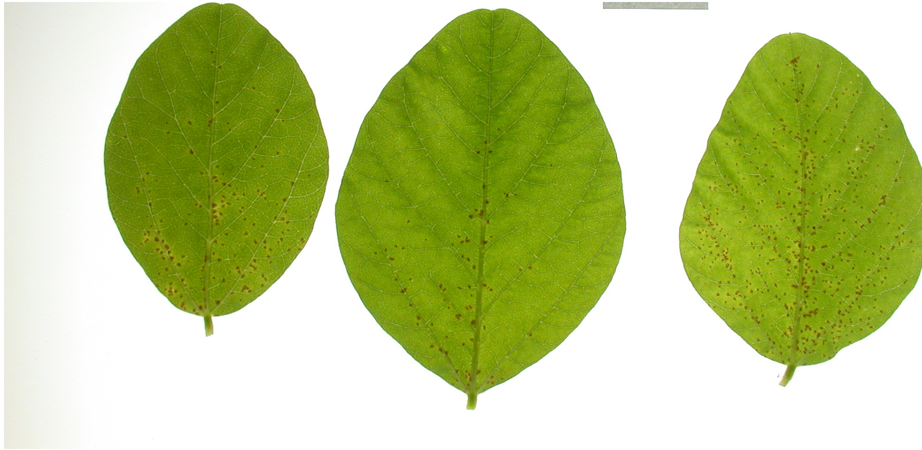
Reference:

Faye, L., and M.J. Chrispeels. 1985. Characterization of N-linked oligosaccharides by affinoblotting with Concanavalin A-peroxidase and treatment of the blots with glycosidases. Anal. Biochem. 149, 218–224.

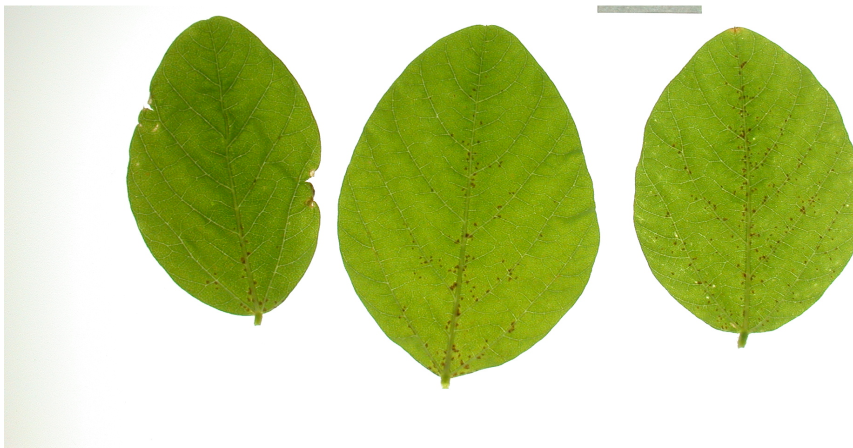
Control – 11 dpi

rPHEP 369 - 1μg - 11 dpi

Fig. S4 – Soybean leaves, of 21-day old greenhouse-grown cultivar Williams 82, were vacuum infiltrated at 15psi for 5 minutes with 1ug of rPHEP 369 protein suspended in 10uL of 0.1X PBS with 0.02% (v/v)Silwet L-77. Control plants lacked the rPHEP 369 protein. Two droplets of infiltrate were applied to each leaflet on one fully expanded trifoliate. Plants were monitored daily for phenotypic reactions to 11 days.



PHEP 369 Trifoliate - 1μg



Control Trifoliate

Fig. S5 – Soybean plants, greenhouse-grown cultivar Williams 82, were treated as described in the legend of Fig. S4 with rPHEP 369 protein or control infiltrate. After 1 day, plants were challenged with *Phakopsora pachyrhizi* TW 72-1. Inoculations were performed as described in the materials and methods section. Plants were monitored every other day for phenotypic response and infection. Leaves were detached and imaged at 15dpi.

	Number of Germinated Spores		
Treatment	**Slide A**	**Slide B**	**Slide C**
PHEP 369 Preimmune	48	46	43
PHEP 369 Sera	46	29	44

Table S1. Number of spores of *P. pachyrhizi* TW 72-1 that germinated on a glass slide following treatment with PHEP 369 preimmune or IgG sera. Freshly tapped TW 72-1 spores are hydrated for 1 hour and diluted in 0.1X PBS to make a working suspension. A quantity of approximately 100 spores are delivered to a glass slide in 50uL of a 1:10 dilution of preimmune or sera in 0.1X PBS. Slides are incubated in a dark, moist chamber overnight at room temperature. Germination counts are performed at 20X magnification.

	Number of Adhered Spores		
Treatment	**Leaf A**	**Leaf B**	**Leaf C**
PHEP 369 Preimmune	161	202	203
PHEP 369 Polyclonal Ab	153	167	201

Table S2. Number of spores of *P. pachyrhizi* LA 04-1 that adhered to a detached leaf following treatment with PHEP 369 preimmune or polyclonal Ab. Frozen LA 04-1 spores are heat shocked for 5 minutes at 40°C, then hydrated overnight. Spores are diluted into 0.1 PBS to make a working suspension. An quantity of approximately 200 spores are delivered to a detached leaf in 100uL of a 1:500 dilution of preimmune or sera in 0.1X PBS. Slides are incubated in a dark, moist chamber for 4 hours at room temperature. Adhesion counts are performed at 20X magnification.