# Pathogenicity of Rhizoctonia Solani to Aquatic Plants

B. G. Joyner and T. E. Freeman

Graduate Research Assistant and Professor, Plant Pathology Department, University of Florida, Gainesville 32601.

Based on portions of an M. S. thesis, University of Florida.

This investigation was funded by U. S. Army Corp Contract No. DACW 73-71-C002, and supported by the Florida Department of Natural Resources Contract No. 3 and by the United States Department of the Interior, Office of Water Resources Research, as authorized under the Water Resources Research Act of 1964.

Florida Agricultural Experiment Station Journal Series No. 4640.

Accepted for publication 12 December 1972.

ABSTRACT

A *Rhizoctonia solani* isolate (RhEa) from diseased anchoring hyacinth (*Eichhornia azurea*) in Panama was pathogenic to several aquatic plants, particularly to water hyacinth (*E. crassipes*) and water lettuce (*Pistia stratioites*). Penetrating hyphae of RhEa grew from infection cushions through the stomates of water hyacinth leaves. Only the emersed portions of water pennywort (*Hydrocotyle umbellata*) and water hyacinth were infected by RhEa. Another *R. solani* isolate (14011) infected both submersed and emersed portions of these two plants. Disease, caused by RhEa, in water hyacinth was severe at 28 C, but as temperature was increased to 32 C, disease severity decreased and little or no damage was detected. Disease severity in water hyacinth caused by another *R. solani* isolate (H287) was the same at both 28 and 32 C.

Phytopathology 63:681-685

*Additional key word:* biocontrol.

---

In 1970, a study of the diseases of aquatic plants was initiated by the Plant Pathology Department, University of Florida, with the idea that certain pathogens might have potential as biocontrols of noxious species; a subject recently reviewed by Zettler & Freeman (22). Notable among the pathogens found was a species of *Rhizoctonia* isolated from blighted anchoring hyacinth (*Eichhornia azurea*) in Panama and subsequently found to be pathogenic to water hyacinth, *E. crassipes* (6). This isolate was identified on the basis of mycelial and sclerotial characteristics, as *R. solani* (9).

There have been previous reports of *Rhizoctonia* spp. occurring on water hyacinth. In 1933, *Hypochnus sasakii*, now considered synonymous with *R. solani* (17), was found to infect water hyacinth in Taiwan (10). Later, *Corticium solani* was reported from water hyacinth in India (13, 14). In addition, Bourn & Jenkins (2), reported *R. solani* to be responsible for the destruction of important duck food plants such as *Potamogeton pectinatus, P. perfoliatus, Ruppia maritima, Vallisneria spiralis,* and *Najas flexilis* in Virginia and North Carolina. *R. solani* also infects alligator weed (*Alternathera philoxeroides*) (10, 19) and the related *A. sessilis* (13).

It was the objective of this work to compare the pathogenicity of the Panama isolate (RhEa) and other isolates of *R. solani* to water hyacinth and other aquatic plants to determine if pathogenic variation exists that may be useful in the utilization of this fungus as a biocontrol of noxious species.

MATERIALS AND METHODS.—Six *R. solani*

isolates were obtained from the Florida Type Culture Collection maintained by the Division of Plant Industry, Florida Department of Agriculture and Consumer Services in Gainesville, Florida. These were FTCC 375 isolated from *Cocos nucifera*, FTCC 17 from *Butia capitata*, FTCC 73 from *Macadamia ternifolia*, FTCC 592 from *Carissa grandiflora*, and isolates we have designated, BJ1 from *Rhododendron* sp., and BJ2 from *Carissa* sp. One isolate (H287) from *Vigna sinensis*, was obtained from T. A. Kucharek (Department of Plant Pathology, University of Florida). Two isolates were obtained from the American Type Culture Collection (ATCC): *R. solani*, ATCC 14011 (15, 16), and *Thanatephorus cucumeris*, ATCC 16987 (5). One isolate (AG-4) was obtained from N. A. Anderson, Department of Plant Pathology, University of Minnesota (1, 7). Also used was an unidentified *Rhizoctonia* (112), isolated during the course of this study from turions (dormant propagating structures) of hydrilla (*Hydrilla verticillata*). Pathogenicity of these isolates was compared with that of the Panama isolate (RhEa) on water hyacinth and other aquatic plants. Cultures of the fungi used in this study are in the Florida Type Culture Collection.

Aquatic plants were obtained from lakes or rivers in North and Central Florida. The following submersed aquatic plants were included: Eurasian watermilfoil (*Myriophyllum spicatum* L.), parrot feather (*M. brasiliense* Comb.), hydrilla [*Hydrilla verticillata* (L.F.) Casp.], coontail (*Ceratophyllum demersum* L.), vallisneria (*Vallisneria* sp. L.), and Brazilian elodea (*Egeria densa* Planch.). The emersed aquatic plants included were: pickerel weed (*Pontederia lanceolata* Nutt.), alligator weed [*Alternathera philoxeroides* (Mart.) Griseb.], salvinia (*Salvinia rotundifolia* Willd.), common duckweed (*Lemna minor* L.), water pennywort (*Hydrocotyle umbellata* L.), frogbit [*Limnobium spongia* (Bosc.) Steud.], water lettuce (*Pistia stratioites* L.), and water hyacinth [*E. crassipes* (Mart.) Solms]. Plants tested represent nine families: Amaranthaceae, Araceae, Ceratophyllaceae, Daucaceae, Haloragaceae, Hydrocharitaceae, Lemnaceae, Ponterderiaceae, and Salviniaceae.

Several methods were used to inoculate aquatic plants: (i) Blocks (0.5 $cm^2$) cut from leading edges of 2-to 3-day-old cultures growing on Difco PDA supplemented with 5g/liter of yeast extract (PDAY) were placed on the leaves, stems and roots. (ii) The fungus was grown on Czapek-Dox broth plus 5g/liter yeast extract (CDY) or without yeast extract (CD) for 5-7 days. Mycelial mats were collected, washed in distilled water, and chopped in a Waring Blendor for 2 min. Various concentrations of the resulting suspension were either sprayed onto plant foliage or poured into the container in which submersed plants were growing. (iii) Hydrilla was inoculated in a manner similar to that used by Bourn & Jenkins (2) for inoculating aquatic plants with *R. solani*. With this method, either sclerotia or chopped mycelia from 5-to 10-day-old PDAY cultures were mixed with washed builder's sand which was placed in 250-ml specimen jars. Hydrilla plants or turions were then planted in the sand and distilled water added to cover the plants.

Inoculated emersed aquatic plants were maintained in clay pots lined with plastic bags, beakers or gallon jars filled with aged tap water. Moist plastic bags were used to cover inoculated plants in pots, whereas either aluminum foil or petri dish lids were used to cover the other containers. Inoculated submersed plants were maintained in either 100-ml test tubes or 300-ml specimen jars containing distilled water.

To study the effect of temperature on infection of water hyacinth by RhEa, plants or excised leaves were inoculated with mycelium in agar blocks (1 $cm^2$). Inoculated plants or excised leaves were kept in growth chambers at 20, 25, 28, 30 and 32 C, with 12 hr/day of light (900-980 ft-c) from incandescent and fluorescent sources. In another experiment, noninoculated plants were grown in growth chambers at temperatures of 20, 28, 30, and 32 C for 12 days. These plants were then inoculated and placed in growth chambers at 20, 28, 30, and 32 C so that at each temperature there were plants that had been grown for 12 days at 20, 28, 30, and 32 C.

To determine mode of penetration, portions of hyacinth leaves, selected 40 hr after inoculation with RhEa at 28 and 32 C, were killed and fixed, imbedded in paraffin, sectioned, and stained with safranin and fast green (8). Epidermal strips and pieces of fresh leaf material cut from infected (RhEa and H287) hyacinth leaves were stained with acid fuchsin-lactic acid prior to examination (3). The colloidin technique (20) was used to study stomatal opening and penetration of epidermis. This technique was also used to check for stomatal opening of noninoculated leaves grown at temperatures of 28 and 32 C.

Ability of isolates of *R. solani* to infect emersed and submersed parts of test plants was determined by two methods. In the first method, water pennywort growing in specimen jars was inoculated with isolates RhEa, 14011, 16987, AG-4, and 112. Agar blocks on which the mycelium was growing were pinned to the pennywort stems by wire so that one block was above water and another below. Inoculated plants were kept at room temperature (20-25 C) on laboratory benches. In the second method, water hyacinth plants growing in about a quart of distilled water in gallon jars were inoculated with the same isolates by placing an agar block with mycelium on the inflated petiole above the water and a similar agar block on the petiole below the water. Inoculated plants were kept on laboratory benches at 20-25 C.

RESULTS.—Results of inoculations of submersed aquatic plants were occasionally difficult to interpret because noninoculated control plants sometimes declined also. However, submersed roots of *Vallisneria* obviously became diseased after inoculation with RhEa. Infected roots turned black, whereas those of noninoculated plants remained white. RhEa was recovered from the blackened roots. Eurasian watermilfoil and hydrilla also became

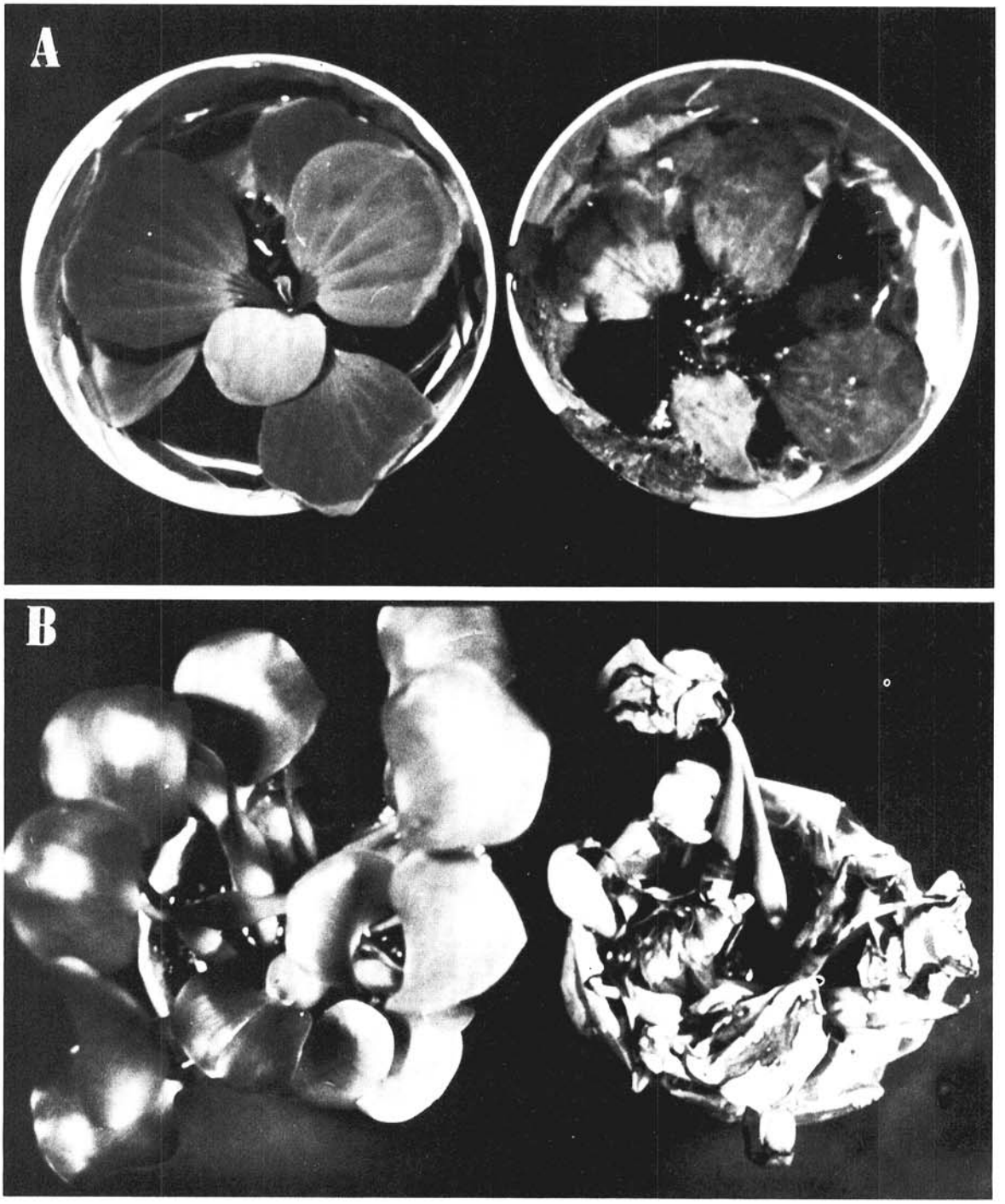


**Fig. 1.** Disease damage on water lettuce (A) and water hyacinth (B) inoculated with RhEa isolate of *Rhizoctonia solani* (right) in comparison with noninoculated check plants (left).

diseased after inoculation with RhEa, and the fungus was recovered from the diseased tissues. The symptoms initially appeared on submersed portions as dark brown lesions on the stems followed by chlorosis and subsequent death of the leaves. When hydrilla sprigs and germinating turions were inoculated with sclerotia of RhEa, no infection was visible. After 4 months, the sclerotia were still intact, but had not germinated. However, these sclerotia germinated one day after being removed from the

water and placed on water agar. Parrot feather became infected when inoculated with RhEa, as well as with the isolates 375, 17, BJ1, 73, BJ2, 14011, AG-4, and 16987, but infection occurred only in tips of shoots that protruded above the surface of the water. Diseased stems were necrotic and young shoots became chlorotic and collapsed after being infected. New shoots frequently developed below the points of infection. Infection was most severe following inoculations with isolates RhEa, 14011 and AG-4. Brazilian elodea and coontail never showed evidence of infection by any isolate.

Every emersed aquatic plant inoculated with RhEa became infected, and RhEa was readily isolated from diseased tissue. At no time did noninoculated check plants appear diseased. Water hyacinth and water lettuce were the most susceptible to the infection by RhEa (Fig 1). This was evidenced by severe secondary infection from the radiating hyphae which frequently killed the plant. Excised leaves of hyacinth, inoculated with RhEa at 28 C, exhibited necrotic lesions in less than 24 hr, whereas inoculated entire plants were similarly affected 24-48 hr after inoculation. Initial symptoms on water hyacinth, regardless of the method of inoculation used, are distinct lesions with tannish brown centers [10.0 YR 6/4, according to Munsell color system (11)] and darker margins. Lesions are usually oval, but may be irregular in shape. In 48 hr, the lesions are about 2-2.5 cm in diameter when inoculated with mycelium in 1-$cm^2$ agar blocks. Secondary infection resulting from growth of the mycelium occurs away from the initial lesions and within a few days the entire upper portion of diseased plants collapse (Fig. 1A). RhEa inoculated roots of water hyacinth remained healthy. Symptoms occurred in water lettuce within 3-4 days after inoculation. Lesions were smaller and more irregular than those on water hyacinth. Within 7-10 days all emersed portions of the plant collapsed (Fig. 1B).

Symptoms induced by RhEa in other aquatics with large leaves (frogbit and pickerel weed) were similar to those on water hyacinth and water lettuce. However, secondary spread from the initial lesion was not as evident as on water hyacinth and water lettuce. On small-leaf aquatic plants (alligator weed, water pennywort, duckweed, and salvinia), the irregularly shaped lesions were smaller and less distinct than on water hyacinth and water lettuce.

Isolates 14011 and H287 were the most virulent of the other isolates tested. Their pathogenicity on water hyacinth was about the same as RhEa. Lesions caused by H287 and 14011 are darker brown (10.0 YR 4/4) than those caused by RhEa. Both of these isolates will cause complete blighting and collapse of water hyacinth.

Temperature affects the severity of disease development in water hyacinth inoculated with the RhEa. Infection was severe 48 hr after inoculation at 28 C, but only slight infection occurred at 30 C and no infection was evident at 32 C. The most severe infection always occurred at 28 C, regardless of the temperature at which the plants had been grown prior to inoculation. However, isolate H287 infected water hyacinth at both 32 C and 28 C. The severity of infection by H287 was the same at both temperatures, and infection occurred in less than 24 hr at both temperatures.

RhEa penetrated via stomates of water hyacinth within 40 hr after inoculation at 28 C, but not at 32 C. Penetration other than through stomates was not observed. Infection cushions always formed over stomata of hyacinth leaves kept at 28 C. However, stomates were open at both 28 and 32 C, and there were no apparent differences in the extent to which they were open.

Infection of water pennywort stems by RhEa occurred only where stems were out of the water. However, isolate 14011 infected water pennywort, both above and below the water level, and could be isolated from both portions of diseased stems. Isolates 16987 and 112 did not infect either portion of stems, whereas isolate AG-4 infected only the portion of the stem above the water level.

When the petioles of water hyacinth were inoculated, RhEa infected only the above-water portion. When a petiole was inoculated below the water, the fungus grew to the top of the petiole where infection occurred (even when air was bubbled into the water, infection by RhEa still occurred only on portions out of the water). Isolate 14011 infected portions of the petiole both above and below the water surface. No infection occurred in water hyacinth inoculated with isolates 112, 16987 or AG-4.

DISCUSSION.—The Panama isolate (RhEa) of *Rhizoctonia solani* was pathogenic to all emergent types of aquatic plants tested; it was extremely pathogenic to water hyacinth and water lettuce. But it only occasionally infected three of the submersed aquatics: Eurasian watermilfoil, hydrilla and *Vallisneria*. It appears that RhEa is either weakly pathogenic to submersed plants or that RhEa loses much of its pathogenic capability under water. Isolate 14011, but not the RhEa isolate of *R. solani*, infected under water portions of both water pennywort and water hyacinth. However, this isolate was nonpathogenic on submersed aquatic plants.

*Rhizoctonia solani* most commonly penetrates hosts through the intact epidermis from beneath dome-shaped infection cushions but can penetrate directly from lobate appressoria and through openings and wounds (4). Ullstrup (21) reported that certain isolates of *R. solani* entered leaves of China aster through stomates. The RhEa strain of *R. solani* entered hyacinth leaves through stomates from dome-shaped infection cusions that formed over the stomates. Submersed aquatic plants either have no stomates or the stomates are functionless (18). Consequently, *R. solani* may have no avenue of entry, other than wounds, into submersed aquatic plants. The RhEa isolate apparently does not have the ability to penetrate the submersed aquatics, despite the fact that their leaves have a thin cuticle and epidermis (18) that would not appear to present a mechanical barrier.

It is interesting to note that RhEa is very virulent on cucumber at 32 C (6) but does not severely affect water hyacinth at this temperature. Since water hyacinth is severely affected by *Rhizoctonia* isolate H287 at 32 C, it appears that the host-pathogen (water hyacinth-RhEa) interaction at this temperature results in sharply reduced disease development. Temperature did not affect stomatal opening at 32 C and apparently did not influence infection.

The RhEa strain of *R. solani* was pathogenic to aquatic plants in seven families. It also infects many terrestrial plants (9). Nag Raj (12) stated that *R. solani* could be used as a biocontrol of water hyacinth if it did not have such a wide host range. The virulence of the RhEa strain of *R. solani* strongly suggests the possibility of using this fungus to control certain water weeds. However, this pathogen cannot be recommended until it is known whether or not it threatens any species that may play an important role in the ecology of lakes, streams and ponds. At the present time, H287 appears to be the best isolate for further study, since it occurs in Florida naturally and will incite disease development in water hyacinth at a higher temperature than the RhEa strain.

## LITERATURE CITED


1. ANDERSON, N. A., H. M. STRETTON, J. V. GROTH, & N. T. FLENTJE. 1972. Genetics of Heterokaryosis in Thanatephorus cucumeris. Phytopathology 62:1057-1065.
2. BOURN, W. S., & B. JENKINS. 1928. Rhizoctonia disease on certain aquatic plants. Bot. Gaz. 85:413-426.
3. CHRISTOU, T. 1962. Penetration and host parasite relationships of Rhizoctonia solani in the bean plant. Phytopathology 52:381-389.
4. DODMAN, R. L., & N. T. FLENTJE. 1970. The mechanism and physiology of plant penetration by Rhizoctonia solani. p. 149-160. *In* J. R. Parmeter, Jr. [ed.]. Rhizoctonia solani, biology and pathology. University of Calif. Press, Los Angeles.
5. FLENTJE, N. T., R. L. DODMAN, & A. KERR. 1963. The mechanism of host penetration by Thanatephorus cucumeris. Aust. J. Biol. Sci. 16:784-799.
6. FREEMAN, T. E., & F. W. ZETTLER. 1971. Rhizoctonia blight of water hyacinth. Phytopathology 61:892 (Abstr.).
7. GROTH, J. V. 1969. Studies of Heterokaryosis in the genus Thanatephorus. M. S. Thesis, University of Minn., St. Paul.
8. JENSEN, W. A. 1962. Botanical Histochemistry. W. H. Freeman and Co., San Francisco. 408 p.
9. JOYNER, B. G. 1972. Characterization of a Rhizoctonia sp. pathogenic to aquatic plants. M. S. Thesis. University of Florida, Gainesville, Florida. 41 p.
10. MATSUMOTO, T. W., W. YAMAMOTO, & S. HIRANE. 1933. Physiology and parasitism of the fungi generally referred to as Hypochnus sasakii. II. Temperature and humidity relations. J. Soc. Trop. Agr., Taiwan 5:332-345.
11. MUNSELL, Book of Color, Vol I. 1929-1942. Munsell Color Co., Inc., Baltimore, Maryland. Looseleaf. n. p.
12. NAG RAJ, T. R. 1965. Thread blight of water hyacinth. Current Sci. 34:618-619.
13. NAG RAJ, T. R., & K. M. PONNAPPA. 1967. Some interesting fungi of India. Commonwealth Inst. Biol. Control Tech. Bull. 9:31-43.
14. PADWICK, G. W. 1946. Notes on Indian fungi IV. Commonwealth Mycol. Inst., Mycol. Paper 17:1-12.
15. PAPAVIZAS, G. C. 1965. Comparative studies of single basidiospore isolates of Pellicularia filamentosa and Pellicularia praticola. Mycologia 57:91-103.
16. PAPAVIZAS, G. C., & C. B. DAVEY. 1962. Isolation and pathogenicity of Rhizoctonia saprophytically existing in soil. Phytopathology 52:834-840.
17. PARMETER, J. R., JR., & H. S. WHITNEY. 1970. Taxonomy and nomenclature of the imperfect stage. p. 7-19. *In* J. R. Parmeter, Jr. [ed.]. Rhizoctonia solani, biology and pathology. University of Calif. Press, Los Angeles.
18. SCULTHORPE, C. D. 1967. The biology of aquatic vascular plants. S. E. Martin's Press, New York. 610 p.
19. SIMS, A. C., JR. 1956. Factors affecting basidiospore development of Pellicularia filamentosa. Phytopathology 46:471-472.
20. TUITE, J. 1969. Plant pathological methods: fungi and bacteria. Burgess Publishing Co., Minneapolis, Minn. 239 p.
21. ULLSTRUP, A. J. 1936. Leaf blight of China aster caused by Rhizoctonia solani. Phytopathology 26:981-990.
22. ZETTLER, F. W., & T. E. FREEMAN. 1972. Plant pathogens as biocontrols of aquatic weeds. Annu. Rev. Phytopathol. 10:455-470.